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TABLE OF CONTENTS

Cultivation of wild forms of the mushroom Psalliota bispora (Lge.) Schäffer & Möller ..... I. WAHL 793

Metabolic resistance to plant diseases ..... C. SEMPIO 799

Studies on the parasitism of Rhizoctonia solani Kuehn on soybeans. M. G. BOOSALIS 820

Red node, a virus disease of beans. H. REX THOMAS AND W. J. ZAUMEYER 832

Yellow stipple, a virus disease of bean. W. J. ZAUMEYER AND H. REX THOMAS 847

Factors influencing invasion of potato by Erwinia carotovora. JOHN P. HOLLIS AND ROBERT W. GOSS 860

Report and abstracts of the seventh annual meeting of the Potomac Division, The American Phytopathological Society 869

Phytopathological Notes

Oat rust urediospores exceed host tissue in cold resistance. JOHN W. OSWALD AND COIT A. SUNESON 875

Plaque formation by Xanthomonas pruni bacteriophage. A. EISENSTARK AND H. H. THORNBERRY 876

Announcement 878

CULTIVATION OF WILD FORMS OF THE MUSHROOM PSALLIOTA BISPORA (LGE.) SCHÄFFER AND MÖLLER

I. WAHL[1]

(Accepted for publication January 23, 1950)

Several workers have pointed out that the common field mushroom, *Agaricus campestris* (L.) Fr., differs from the cultivated forms (11). Atkinson (1) was the first to discover that the cultivated variety is mainly 2-spored (with some 3-spored basidia), whereas the wild field mushroom is 4-spored. The cultivated forms possess marginal cystidia on the gills (6) while the wild field mushroom lacks them, and the mycelium of the latter does not grow in fermented manure (3). Despite these facts, it is customary (5) to consider all the cultivated forms of the genus Agaricus as varieties of the species *Agaricus* (*Psalliota*) *campestris* (5); and even as late as 1943 Kligman (4), although aware of the fundamental differences between the common field mushroom and the cultivated mushrooms, nevertheless designated the latter as *Agaricus campestris* Fr. (var. *bisporus*).

Wild specimens of a 2-spored mushroom of the genus Agaricus have been found in both Europe and America, and these have been looked upon as possible sources of the cultivated races (3). At Cornell University Atkinson (1) collected 2-spored specimens "resembling certain cultivated varieties." Buller (2, p. 15) found 2-spored fruit bodies on manured grounds at the University of Manitoba. The brownish, scaly pilei differed considerably from those of the common field mushroom. Sinden (personal letter) found in California 2-spored, brown, scaly, rather small mushrooms fruiting prolifically on manure compost.

None of these investigators has attempted to set up a systematic nomenclature for this fungus. In Europe, on the other hand, a more complete description with systematic designation was given by Lange (6) and Schäffer & Möller (9). Lange (6) coined for the 2-spored fungus the name *Psalliota hortensis* var. *bispora* and in 1939 he elevated the variety to a separate species, *Psalliota hortensis* (7). Lange attributed systematic significance to the marginal cystidia in *P. hortensis* and emphasized also that the natural habitat of this species is rich, loamy soil, whereas *P. campestris* has 4-spored basidia, lacks cystidia completely, and is ordinarily restricted to fields. Schäffer and Möller (9) presented a rather detailed diagnostic description of the 2-spored species and named it *Psalliota bispora*. According to their information the mushroom appears in groups on compost piles or pastures.

[1] The writer is greatly indebted to Dr. C. M. Christensen for the suggestion of the problem and his constant interest and helpful advice throughout the investigation and in preparation of the manuscript. He also desires to express his sincerest appreciation for inspiring criticism and valuable assistance to Dr. E. C. Stakman, Dr. L. Dosdall, and to the Editorial Committee of the Department of Plant Pathology and Botany of the University of Minnesota.

As to the origin of the cultivated races, the following provision was stipulated (9): "Ob diese Wildart als Stammform aller zweisporigen Kulturrassen anzusprechen ist—diese weichen oft stark ab!—soll hier dahingestellt bleiben." Dealing with the same question, Cayley (3) is inclined to believe that the 2-spored "form or species" collected in America or one very similar to it found in England "may be the origin of the cultivated varieties." So far as is known, the physiology of the 2-spored wild mushroom has not been investigated, although such a study might aid in obtaining new forms for cultivation and shed more light on the problem of derivation of the present cultivated mushrooms. For this reason, the following study was undertaken with specimens growing wild at University Farm, St. Paul, Minnesota.

Since the collected sporophores differ among themselves morphologically, and at least two types can readily be distinguished, a detailed description seems pertinent.

TYPES OF SPOROPHORES COLLECTED

The brownish, scaly type (Fig. 1, A) was collected by Mr. M. B. Moore on a loamy, bare spot between field plots. According to available information this place had not been treated with manure for at least several years. The color of the mature pileus varies between Wood Brown and Avellaneous, according to Ridgway (8). Mature caps are 5–9 cm. in diameter, convex when young, gradually becoming flat. The surface is partially covered with scales (Fig. 1, A) more pronounced toward the margin, while the central part usually remains smooth. The margin is lighter in color, expands beyond the edge of the gills, is framed with a rim consisting of fragments of the veil, and is sometimes curled backwards when fully expanded. The flesh is white, compact, and firm, turning slightly brownish when cut (in mature specimens); gills are crowded, free, pinkish when young, purplish brown in more advanced stages. Cystidia are marginal, $23–32 \times 5.6–10.1\ \mu$; basidia are $23–30 \times 5.2–8.5\ \mu$; basidiospores are broadly ovate, $5.5–10.3 \times 5.3–7.5\ \mu$. The annulus is near the middle of the stem and disappears in mature specimens. The stem is solid, stuffed, 4–5 cm. long and 1.2–1.5 cm. thick. The surface of the stem is smooth or covered with delicate scales or fibrillose patches when mature.

The creamy white type (Fig. 1, B) was collected by Dr. C. M. Christensen from a manure pile at University Farm (a similar specimen was sent to Dr. L. Dosdall from an unknown source). The pileus is ordinarily 5–8 cm. in diameter varying in color from white to Vinaceous Buff (8), smooth or fibrillose at the center. In some specimens the marginal part is broken into fibrillose patches or is slightly scaly. The margin extends beyond the gills and is curled backwards in fully grown mushrooms. The flesh is white, firm, turning slightly brown when cut. The gills in young sporophores are pinkish and become purplish brown in older specimens. The cystidia are marginal, $23–35 \times 5.9–11.5\ \mu$; basidia are $23–32 \times 5.4–6.5\ \mu$;

basidiospores are 5.3–10 × 5.1–7.0 μ. The annulus is located near the middle of the stem and disappears in older sporophores. The stem is solid, stuffed, 2–3 cm. long before the veil is torn away; 8–9 cm. long and 1.2–1.5 cm. wide when fully grown. While the brown scaly type fits well the descrip-

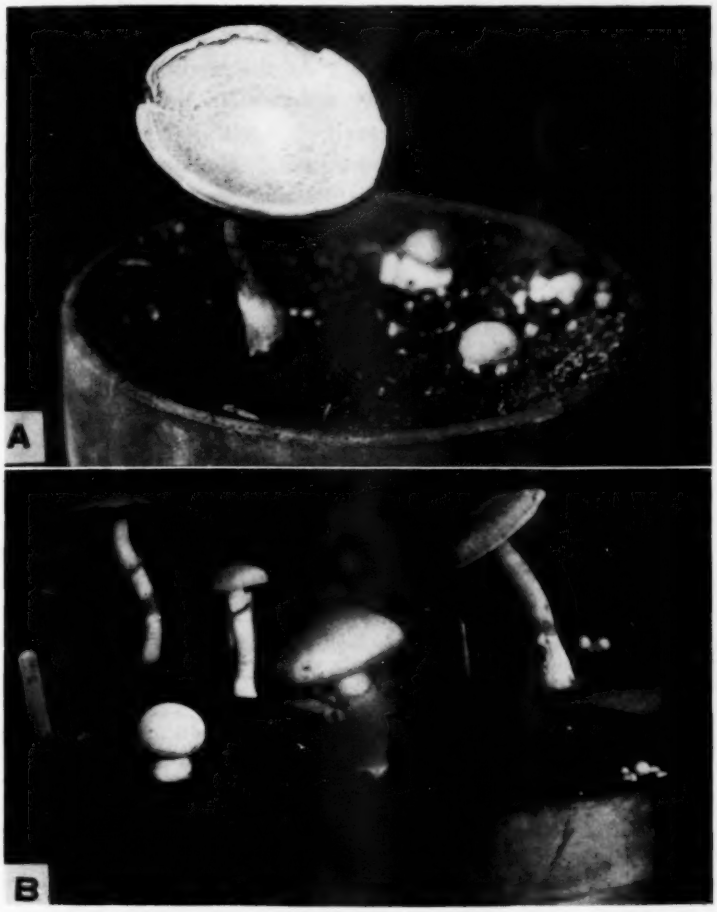

FIG. 1. Two types of sporophores of *Psalliota bispora* (Lge.) Schäffer and Möller: A. The brownish, scaly mushroom. B. The creamy white mushroom.

tion given by Schäffer and Möller (9), the creamy white type diverges in certain morphological features, as in the relation between the length of the stem to the diameter of the cap. According to Schäffer and Möller, the stem is equal to or shorter than the diameter of the cap, whereas in the creamy white mushrooms the stem is somewhat longer and consequently the sporophore is slender. For this reason, the creamy white mushroom might be considered as a separate variety of the species *Psalliota bispora* (Lge.) Schäff. and Möll.

CULTIVATION EXPERIMENTS

Multisporous and tissue cultures of the brown scaly mushroom were

secured and grown on potato-dextrose agar (Fig. 2). Since the primary object of preliminary experiments was to obtain general information regarding cultivation under artificial conditions, no attempt was made to prepare single spore cultures. The spores which were allowed to drop freely from the hymenium germinated readily after 5–10 days on potato-dextrose agar. The mycelium grew vigorously and in 2–3 weeks covered all the substrate in 125-cc. Erlenmeyer flasks. At the beginning the culture was flat, but became gradually more fluffy towards the margin of the medium. In sterilized grain the fungus grew well, and within 2 weeks a spawn with abundantly growing mycelium was obtained. The spawn was scattered in 5- or 6-in. pots containing fermented horse manure. The manure used was allowed to ferment naturally for 2 days, then was kept for 12–14 days in ovens at 135°–140° F.

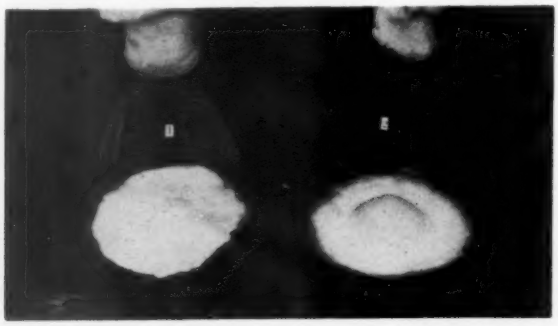

FIG. 2. Mycelial cultures of *Psalliota bispora* (Lge.) Schäffer and Möller. 1. Colony of the brown, scaly fungus. 2. Colony of the creamy white mushroom after treatment with uranium nitrate.

The mycelium developed vigorously in this compost. In 12–14 days the substrate became thoroughly permeated with the hyphae and ready for casing. During all this time the pots were exposed to 70° F. After casing, the temperature was lowered to 57°–60° F. The first pilei were produced 27 days from the time of spawning. The brown scaly type yielded 2 to 3 times as many fruit bodies as a brown commercial race cultivated under the same conditions. Successive "breaks" occurred at intervals of 8 to 10 days. At 60° F. the full development of the pileus ordinarily required 8 to 11 days.

Multisporous and tissue cultures of the creamy white mushroom were easily obtained and grown on potato-dextrose agar. Cultures obtained by both methods grew slowly. Fifty hyphal tips were cut from the original cultures, isolated, and subcultured, and from these, three vigorously developing cultures were selected. Transfers from these three cultures were plated on potato-dextrose agar containing uranium nitrate $UO_2(NO_3)_2$, $6H_2O$ at the rate of 0.1–0.75 gm. per liter, with the purpose of producing fast growing mutants—as had been reported with a brown cultivated race (10). The first subcultures remained unchanged. After 3 months of con-

tinuous transferring (at 2-week intervals), however, in a few cases fluffy abundant mycelium appeared (Fig. 2). These new cultural characters remained constant even when the culture was brought back to plain potato-dextrose agar. Grain spawn from mycelium treated and nontreated with uranium nitrate was prepared. The fluffy cultures grew much better than those not treated with uranium nitrate. After 14 to 17 days the spawn was inserted in 5-in. pots with fermented horse manure. At the end of 6 weeks the first fruit bodies began to appear. The pilei produced by both types of mycelium did not differ noticeably; however, in the preliminary experiment the mycelium treated with uranium nitrate yielded the first sporophores 3–5 days earlier than the nontreated ones. This conclusion is preliminary only and requires further confirmation.

DISCUSSION

Two different types of the 2-spored wild mushrooms were collected at University Farm, St. Paul, Minnesota: the brownish scaly and the creamy white fibrillous types. The latter diverges in certain morphological properties from the description given by Schäffer and Möller (9) for *Psalliota bispora* (Lge.) and it is suggested that it may be considered a separate variety of *Psalliota bispora* (Lge.) Schäff. and Möll.

It seems apparent that the 2-spored wild mushrooms are morphologically and physiologically closely related to the cultivated races and the hypothesis that at least some cultivated races derive from the 2-spored wild mushroom form gains additional substantiation. As in the experiments with cultivated mushrooms (10), uranium nitrate was an effective means of increasing the rate of mycelial growth, although it was not established whether the induced characters are retained in passage through the sporophore stage.

SUMMARY

The cultivated mushroom differs from the wild *Agaricus campestris* Fr. in that the cultivated form has 2 spores per basidium, has cystidia on the gill margins, and is able to grow in manure fermented at high temperatures.

A wild 2-spored *Agaricus* sp. occurs in North America and this presumably is identical with or very similar to the *Psalliota bispora* (Lge.) Schäff. and Möll. described by various European workers.

Two morphological types have been distinguished among the wild 2-spored mushrooms of the species *Psalliota bispora* (Lge.) Schäff. and Möll. collected at the St. Paul Campus of the University of Minnesota. One has a brown, scaly cap and a short stem, the other a creamy white, fibrillous cap and a long, slender stem.

In the 2-spored creamy white mushroom the stem is longer than or equal to the diameter of the cap, whereas in the type species of *Psalliota bispora* the stem is equal to or shorter than the diameter of the cap. The writer considers the creamy white form a variety of the species.

Multisporous and tissue cultures of the brown scaly mushroom grew vigorously in manure fermented at high temperature. This fungus is particularly fertile and fruits approximately 30 days after spawning.

Some of the multisporous and tissue cultures derived from the creamy white type have produced, on potato-dextrose agar plus uranium nitrate 0.1–0.75 gm. per liter, abundant mycelium, subcultures of which developed readily on potato-dextrose agar and yielded sporophores in horse manure beds approximately 6 weeks after spawning.

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METABOLIC RESISTANCE TO PLANT DISEASES

C. SEMPIO[1]

(Accepted for publication December 20, 1949)

Among the many resistance mechanisms exerted by plants against their parasites, there are some of a strictly metabolic type (22, 23, 24). Fundamentally, every resistance mechanism is linked, more or less indirectly, to a specific type of metabolism. Linked thus to the metabolism of the plant is the creation of barriers of suberous cells which tend to restrain mechanically the spread of the parasite; likewise the formation, within the host cell, of substances injurious to the development of the pathogen (for instance, phenols); or the capsuling of the haustoria by means of the protoplasm in the attacked cell; or the pseudophagocytic action exerted by the nucleus of the invaded cell on the haustorium of the attacking agent. There are cases, however, where a change in the rate of metabolism must be considered in itself, and not by its consequences, as an immediate and definite manifestation of defense.

Consequently, to the metabolic type of resistance we should ascribe all the protective reactions which the plant effects solely by means of a change in its metabolism, since, in itself, this change constitutes a more or less effective defense.

From what is known or can now be supposed, from experiments on the inmost nature of these defensive processes (so closely linked to cellular life), it is believed that they are realized in two ways: 1) by a change in the balance of the relations normally existing in the intensity of the principal functions of the host (photosynthesis, glycolysis, respiration), generally with definite prevalence of the synthetic process or with definite accentuation of the glycolytic power; 2) by the substitution—in the course of anabolism and catabolism—for some specific intermediate compounds easily assimilable by the parasite, of others, their isomers, well utilized by the plant but assimilable only to a small extent or not at all by the parasite. In this second case, however, we are still in the induction and hypothesis stage, although experimental data are not wanting which might serve as a basis and guide for future investigations.

Since, for the greater part it is a question of the ratio of intensity between photosynthesis, glycolysis, and respiration, for brevity these processes will be indicated by the letters P, G, and R.

The P, G, and R values which will be given throughout are to be taken as always relating to the ratio found in the respective controls, arbitrarily considered as equivalent to 1 : 1 : 1. The P-G-R values refer to the averages of the results obtained.

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The intensity of parasitic attack has been evaluated (9) at degrees rising from 0 to 5. The numbers given in the text represent the mean of the intensities of attack encountered in the various experiments.

The metabolism experiments for ascertaining the rise or fall in the metabolic resistance of the tissues of the host are always carried out, for obvious reasons, on healthy plants (15, 18, 20).

CHANGES IN METABOLIC BALANCE DURING DISEASE DEVELOPMENT

Turning briefly to the experimental data available on the first way of attaining protection by metabolic resistance, certain experimental facts, with special reference to the metabolism of the "wheat-*Erysiphe* complex," have been ascertained (16, 17, 21).

Wheat plants infected with the conidia of *Oidium moniloides* definitely increased, during the first 2 or 3 days of parasitic attack, the intensity of the photosynthetic rate; the stimulation was appreciable, because generally photosynthetic intensity rose during the first period of parasitism to almost double the normal intensity (Table 1). In the following days this activity decreased rapidly, falling below normal; it rose again at the time of differentiation of conidiophores, then gradually fell to very low values at the end of the parasitic cycle (Table 1).

TABLE 1.—*Photosynthetic activity of leaves of Virgilio wheat infected by Oidium moniloides*

Number of days after inoculation	Sunlight and temperature during experiment; and duration of experiment			Milligrams CO_2 fixed by 1 gm. dry matter in 10 liters of air[a]		CO_2 fixation by infected plants if value of 100 is given to healthy plants
				Healthy leaves	Infected leaves	
First leaf		°C.	*Hours*			
2	Clear	16–22	6.50	24.07	41.26	171.4
4[b]	Cloudy	15–16	6.50	20.65	24.30	117.7
6[c]	Clear	13–17	7.50	27.05	18.07	66.7
8[d]	Clear	14–20	8.00	19.30	26.76	138.7
11[e]	Cloudy	16–20	6.50	17.88	22.03	123.2
14	Clear	16–23	4.25	20.07	9.19	45.7
First and second leaves						
1	Clear	21–27	6.50	5.96	8.88	149.0
3[c]	Clear	19–29	7.00	8.47	7.94	93.7
5[d]	Cloudy	17–20	7.00	7.08	13.76	194.4
7[e]	Clear	14–26	7.25	7.56	11.10	146.8
10	Cloudy	17–21	6.25	30.90	29.30	94.8
12	Clear	12–22	7.75	22.55	3.22	14.3

[a] The method used, fixation of CO_2 on barite, was described in (21). In all experiments with only the first leaf and in the experiments at 10 and 12 days after inoculation of the first and second leaves, the atmosphere was enriched with CO_2 to obtain a concentration optimum for assimilation. This explains the high values for fixation of CO_2.

[b] Haustoria.

[c] Mycelium and haustoria well developed.

[d] Conidiophores and conidia formed.

[e] Conidia abundant, disease attack severe.

TABLE 2.—*Respiration of leaves of Virgilio wheat infected by Oidium moniloides*

Number of days after inoculation	Temperature (° C) during the experiment	Ratio: fresh weight / dry weight Mean value in per cent		Average value[a] of Q_{O_2}		Respiration of infected plants if value of 100 is given to healthy plants
		Healthy	Infected	Healthy	Infected	
First leaf						
2	24.6	9.0	9.2	1.73	2.06	119.1
4[b]	23.1	9.7	9.5	0.90	1.18	131.1
6[c]	23.5	8.7	9.6	1.15	2.04	178.2
8[d]	24.5	8.7	9.3	1.20	3.40	283.3
11[e]	24.6	8.7	10.5	0.86	3.73	433.7
14	23.8	9.0	10.2	0.81	3.09	382.1
Second leaf						
1	24.9	11.9	11.3	1.65	2.07	124.8
3[c]	25.3	10.9	11.5	1.61	2.97	184.6
5[d]	23.8	9.5	11.2	1.23	3.94	319.7
7[e]	24.4	9.8	10.5	1.29	4.00	309.7
10	24.4	10.7	11.0	1.25	3.62	290.0
12	24.4	10.0	11.8	1.22	3.18	260.0

[a] Respiration was determined by the Warburg micromanometric method described in (17). Q_{O_2} = mm.[3] of oxygen absorbed by 1 mgm. dry matter in 1 hr.
[b] Haustoria.
[c] Numerous haustoria and mycelium well developed.
[d] Conidiophores and conidia formed.
[e] Conidia abundant, disease attack severe.

TABLE 3.—*Glycolysis in leaves of Virgilio and Mentana wheat infected by Oidium moniloides*

Number of days after inoculation	Temperature (° C) during the experiment	Ratio: fresh weight / dry weight Mean value in per cent		Average value[a] for $Q^{N}_{CO_2}$		Glycolysis of infected plants if value of 100 is given to healthy plants
		Healthy	Infected	Healthy	Infected	
Virgilio—second leaf						
2	24.5	11.7	11.2	0.95	1.17	123.0
4[b]	24.5	10.6	10.8	1.07	1.16	108.7
6[c]	24.4	11.8	11.7	1.05	0.83	79.3
8[d]	24.4	10.7	12.3	0.86	0.60	70.6
11[e]	24.5	11.1	11.4	1.04	0.65	62.1
14	24.4	11.4	13.5	0.80	0.45	55.9
Mentana—first leaf						
1	25.3	8.93	9.38	0.66	0.82	123.9
2	24.8	9.03	9.12	0.68	0.73	107.5
4[b]	25.0	8.33	9.23	0.74	0.72	95.9
6[c]	25.3	8.48	8.90	0.60	0.63	106.6
8[d]	25.3	8.50	9.19	0.74	0.64	87.0
11[e]	25.1	8.70	9.93	0.85	0.58	68.2

[a] Glycolysis was determined according to procedures described in (17). $Q^{N}_{CO_2}$ = mm.[3] of carbon dioxide emitted in 1 hr. by 1 mgm. dry matter in an atmosphere of nitrogen.
[b] Haustoria.
[c] Well developed mycelium.
[d] Conidiophores and conidia formed.
[e] Conidia abundant, disease attack severe.

The rates of respiration and glycolysis also increased during the first days of infection, although these early increases were much less intense than in the case of photosynthesis (Tables 2 and 3). In the following days, rate of respiration continued to rise rapidly until it attained (at the time conidia were differentiating) maximum values 3 to 4 times normal; glycolysis descended fairly rapidly to values below normal, and continued to fall slowly until the end of the attack (Tables 2 and 3). Data were similar for three varieties of wheat: Mentana, Virgilio, and Rieti.

It is important to note that in the initial phase of the disease, P-G-R = 1.71 : 1.23 : 1.19, while in the final phase, P-G-R = 0.45 : 0.56 : 3.82 (Fig. 1).

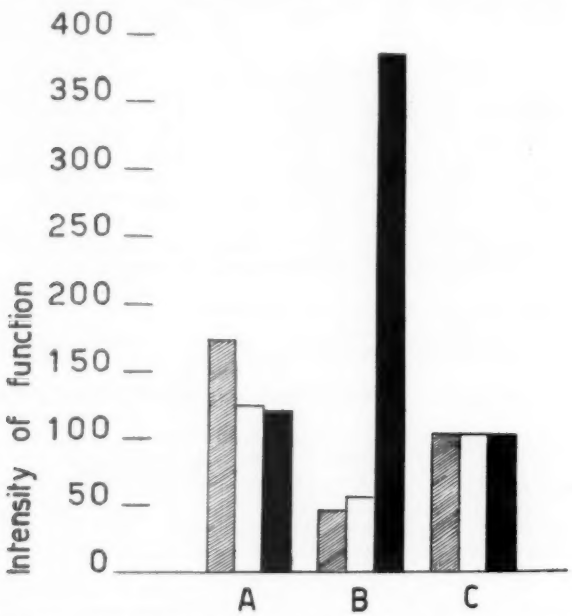

FIG. 1. Proportionate intensities of photosynthesis (shaded bar), glycolysis (white bar), and respiration (black bar) of wheat leaves 2 days after inoculation, in the initial stages (A) and 14 days after inoculation in the final stages (B) of infection with *Oidium moniloides*. Values for healthy control leaves (C) were arbitrarily placed at 100.

If we take 1 as the value of photosynthetic intensity, the P-R ratio was 1 : 0.7 in the initial phase of the disease; it became 1 : 8.5 in the final phase. The photosynthesis-respiration ratios can be calculated from the last column of table 1 and the last column of table 2. The quotient which in the first 2 or 3 days of the disease was considerably over 1 and sometimes as high as 2, gradually dropped close to 0 during the course of the disease, so that at the end of the parasitic cycle, values of 0.12 and even 0.05 were reached. The P-R quotient on the first leaf of Virgilio, during the entire cycle of the disease, was: 1.45–0.90–0.38–0.49–0.28–0.12. The disease, therefore, is essentially a functional disequilibrium which gradually becomes more pronounced during the parasitism.

The stimulus which the three functions, and especially photosynthesis,

undergo during the first few days of disease attack should be considered as a defensive reaction set up by the plant against incipient attack of the parasite.

In plants that are in a state of defense reaction, the greater stimulation of photosynthesis in regard to the other two functions is particularly significant because it is an indication of an accentuation within the green cell of the synthetic processes in comparison with those of demolition, chiefly respiration. It might reasonably be deduced that what occurs is an accumulation of insoluble products (not assimilable by the parasite), as opposed to a relative shortage of soluble products (easily assimilable by the parasite). The percentage equilibrium of the two series of compounds (insoluble and soluble) would be altered in the protoplasm of the host, and parasitism would be affected (10).

The influence of Oidium infection on the respiratory rate of wheat has been studied by other investigators, and in particular by Yarwood (28), Pratt (4), and Allen and Goddard (1, 2). They all found that the respiratory activity increases rapidly after infection. Pratt reported the maximum respiratory intensity 9 days after infection, with values almost triple the normal; after an interval of a few days the respiratory rate decreases. Allen and Goddard found that the respiratory intensity was maximum 6 days after inoculation, with values 3 to 4 times normal. They also noted an appreciable increase in the fermentative power (glycolysis). It is worth noting that, after careful experiments, these researchers came to the conclusion that the considerable increase in respiratory activity is due not to the metabolism of the parasite, but almost exclusively to the stimulus it exerts on the metabolism of the host. Parris (3) showed that *Erysiphe polygoni* did not reduce apparent photosynthesis of bean leaflets until yellowing due to infection had set in; no significant differences in respiration were found between infected and healthy leaves.

RELATIONS BETWEEN THE CHANGE IN METABOLIC BALANCE AND MODIFICATIONS IN RESISTANCE

The dynamic character of these metabolic functions and the importance of their balance is evident when we experimentally alter conditions and sometimes prevent or excite the normal defense reactions within the host plant. In effect, by opportunely changing the environmental and cultural conditions of plants, before or after inoculation, it is possible also to modify variously the metabolism of the host and consequently influence positively or negatively its resistance to a specific parasite.

The Effect of Darkness

Confinement in the dark of infected plants during the first 3 or 4 days

of parasitic attack[2] resulted in greater disease severity than did exposure to normal daylight (11, 12, 18). The intensity of attack was determined 15 to 16 days after inoculation; the following average results were obtained experimentally.

On wheat plants infected with *Oidium moniloides* and kept in the dark for the first 4 days of infection the attack fluctuated between 4.2 and 5; on plants left in daylight during the same period the attack fluctuated between 3 and 4.

On lettuce plants infected with *Bremia lactucae* and kept in the dark for the first 4 days of infection, the attack fluctuated between 4.5 and 5; on plants left in daylight for the same period the attack fluctuated between 3.3 and 4.

TABLE 4.—*Respiration and glycolysis of leaves of Virgilio wheat and young seedlings of Winter Dark lettuce in darkness and in normal daylight*

Treatment and its duration	Temperature (in ° C) during the experiment	Ratio: fresh weight / dry weight Mean value in per cent	Q_{O_2}	$Q^{N}_{CO_2}$
Wheat, second leaf				
Darkness, 2 days	25.2	13.3	1.55	0.66
Control	25.2	15.1	2.53	1.43
Darkness, 3 days	25.2	10.1	1.25	0.59
Control	25.2	12.2	1.48	1.28
Darkness, 4 days	23.5	9.9	1.21	0.59
Control	23.5	11.1	1.79	1.16
Darkness, 5 days	23.8	10.2	1.19	0.63
Control	23.8	11.4	1.75	1.28
Darkness, 6 days	23.5	9.2	1.10	0.52
Control	23.5	11.2	1.59	1.41
Lettuce, 4 to 5 young leaflets				
Darkness, 2 days	23.2	4.1	1.43	0.39
Control	23.2	4.4	2.15	1.12
Darkness, 3 days	23.6	4.0	1.22	0.22
Control	23.6	4.9	1.64	0.70
Darkness, 5 days	23.5	3.8	1.14	0.29
Control	23.5	4.6	1.74	0.95

The differences are distinct; they are also more marked in varieties of medium resistance.

As is known, the consequence of complete darkness is total blocking of photosynthesis; this is evidently the main cause of the more severe attack encountered in plants placed in the dark during the early days of infection.

[2] Numerous investigations have shown that the host-parasite "complex," in the different phases of its cycle, presents a different susceptibility towards the principal environmental factors. Therefore the incubation period was subdivided into three sub-periods: 1) from the 1st to 4th day of incubation (contagion); 2) from the 4th to 7th day of incubation (expansion of mycelium); 3) from the 7th to 10th day of incubation (differentiation of conidiophores) (9, 11). In particular, it was found that darkness may stimulate or depress appreciably the development of the disease, depending on whether it occurs during the first or last sub-period of incubation (11).

For this reason, the initial stimulus of photosynthesis should be interpreted as a defensive reaction.

In addition, on keeping healthy wheat[3] and lettuce plants in the dark for a few days, it was found that this treatment produced a marked disturbance in the glycolysis-respiration balance. Both these functions were depressed, but the depression varied greatly in intensity (Table 4); in fact, the mean G-R ratio of the plants kept in the dark was 0.45 : 0.70 for wheat and 0.32 : 0.70 for lettuce.

Darkness, therefore, modifies considerably the ratio between the functions in question, producing a general but uneven depression in the metabolism, with a most decided predominance of the respiratory process. For wheat—the case most studied—the P-G-R was found to be 0 : 0.45 : 0.70 in the absence of light, while in normal light and during the first few days of Oidium infection, the P-G-R was 1.71 : 1.23 : 1.19. This radical inversion of the functional ratios explains the collapse of the defense power of the plants kept in the dark during the early period of infection.

Effect of Prolonged Exposure to Continuous Artificial Light

Continuous artificial light increased the resistance of wheat to *Oidium* (10, 14, 20), but duration of the exposure to light was a factor in the development of the disease. In these experiments seven varieties of wheat were used: Rieti, Gentilrosso, Mentana, Virgilio, Littorio, Roma, and Frassineto. Wheat seedlings inoculated with *Oidium* were placed in a special white-walled room and exposed to artificial light of approximately 1500 candlepower. One set of plants was exposed to continuous light for 12 consecutive days and another for 4 consecutive days, during the first stages of parasitism; and the controls in the same room were alternately in light for 12 hr. and in darkness for 12 hr. Light was excluded by covering the seedlings with boxes painted black on the inside and provided with special ventilating tubes. At the end of 12 days the three sets of plants were taken to a greenhouse and exposed to daylight; after a week (18 days after infection) the severity of disease attack was rated. In the control plants it ranged from 4.5 to 4.8 for the seven varieties of wheat used; in plants exposed to continuous light for 12 days, disease severity ranged from 0.2 to 1, according to the variety (10, 20). The control plants in this artificial environment were generally more severely attacked than those kept under normal conditions (daylight).

The absence of the normal nocturnal halt in photosynthesis appears to be the chief cause of the very considerable depression in Oidium attack on the plants exposed to continuous light for 12 days. It may be deduced that, under natural conditions, contagion is more likely to occur at night, not so much because of the greater humidity which favors the germination

[3] Tests being carried out on the metabolism of infected plants placed in the dark during the early days of infection indicate that the functional ratios do not differ appreciably from those obtained from healthy plants kept in the dark.

of the conidia, but because, during the night, the functional ratios undergo marked changes, only the respiration continuing to function normally. The living substratum is thus enriched with soluble compounds acceptable to the parasite.

The shorter exposure to continuous light, during the first 4 days of infection, was less effective in reducing disease severity: the rating was approximately 2.5 in Frassineto and 3.5 in the other varieties. This finding confirms the statement on the effect of darkness: during the first period of infection, darkness stimulates the intensity of attack, while continuous light lowers intensity (11, 18, 20).

Prolonged exposure to light did not appreciably affect the percentage of conidia that germinated, nor did it influence the elongation of the mycelium. Consequently, it seems that a direct action by artificial light on the vitality of the conidia is to be excluded. Therefore, functional experiments were begun on the plants treated and on the respective controls, care naturally being taken to prevent Oidium infection.

Glycolytic activity was greater in wheat leaves exposed continuously to light than in leaves of the control plants kept in alternate light and darkness (Table 5). Respiration also was increased perceptibly by exposure to light, but the increase was less than for glycolysis (Table 5) (20).

TABLE 5.—*Respiration and glycolysis in leaves of Virgilio wheat after continuous exposure to artificial light*

Treatment and its duration	Temperature (in ° C) during the experiment	Respiration data		Glycolysis data	
		Ratio: $\frac{\text{fresh weight}}{\text{dry weight}}$ Mean value in per cent	Q_{O_2}	Ratio: $\frac{\text{fresh weight}}{\text{dry weight}}$ Mean value in per cent	$Q^{N}_{CO_2}$
Second leaf					
Light, 5 days	26.3	10.7	1.57	11.2	1.15
Control	26.3	12.1	1.37	12.0	0.95
Light, 6 days	26.5	10.1	1.36	10.5	1.12
Control	26.5	10.3	1.23	10.5	0.71
Light, 7 days	26.4	11.0	1.90	11.0	0.91
Control	26.4	10.9	1.81	11.0	0.85
Light, 8 days	26.6	10.9	1.32	10.8	1.06
Control	26.6	11.2	1.06	11.0	0.80
Third leaf					
Light, 6 days	24.2	9.9	1.58	9.9	1.06
Control	24.2	10.9	1.39	10.7	0.56
Light, 8 days	24.4	9.9	1.52	11.2	1.15
Control	24.4	10.4	1.33	11.7	0.55

In plants exposed to continuous light the G-R ratio was 1.53 : 1.14. It has already been stated that this ratio was 0.45 : 0.7 in plants kept in the dark for a few days. This shows that continuous light, besides subjecting the green tissues to uninterrupted photosynthetic activity, greatly modifies the G-R balance inversely to the effect produced by darkness. The development of the disease was also affected inversely (11, 18, 20). Cytologi-

cal investigations (10, 19) synchronized with metabolic tests indicated that continuous light exerts a marked sensitizing action on the nucleus of the cells invaded by the haustorium. The sensitized nucleus promptly approaches the haustorium, adhering to it and becoming deformed. Following this contact, the haustorium is also deformed and degenerates. This nuclear reaction, which does not occur in the control plants, is rapid and effective. The mechanism by which continuous light induces resistance in wheat, therefore, is not only metabolic, but also nuclear in type. The complete defense is the combination of these different reactions.

Effect of Wilting

Wheat seedlings grown in a humid and shady greenhouse may be easily predisposed to the attack of *Oidium* by sudden exposure to full sunlight for some hours, because the leaves rapidly wilt. It is known that wilted tissues are predisposed to attack (5, 6, 7).

For wheats in such a state of predisposition, there is still a general stimulation of metabolic activity over that in normal plants (Table 6 and 7), but the ratios among the three metabolic functions differ from the ratios obtained for plants having a defense reaction.

In plants predisposed by wilting the P-G-R ratio is 1.23 : 1.43 : 1.60 (25); in plants in a state of defensive reaction, during the first days of Oidium

TABLE 6.—*Photosynthesis in Mentana wheat seedlings subjected to sudden insolation*

Duration of treatment, in hours	Duration of experiment, in hours	Temperature (in ° C) during the experiment	Milligrams CO_2 fixed by 1 gm. dry matter in 10 liters of air		CO_2 fixation by treated plants if value of 100 is given to control plants
			Control	Treated (insolation)	
4	4	19–22	7.11	9.58	134.7
4	4	20	5.72	7.01	122.6
4	3½	22–27	3.74	3.97	106.1

TABLE 7.—*Respiration and glycolysis in second leaf of Mentana wheat exposed to sudden insolation*

Duration of treatment, in hours	Temperature (in ° C) during the experiment	Ratio: fresh weight / dry weight		Q_{O_2}			$Q^{N}_{CO_2}$		
		Control	Treated	Control	Treated	Per cent	Control	Treated	Per cent
3	21.5	14.7	15.1	1.63	2.90	178			
3	21.5	15.0	15.8	1.43	2.79	195			
3	24.2	13.2	18.3	2.15	2.66	124			
3	24.2	14.0	18.0	2.37	3.66	154.5			
7	23.6	14.0	16.0	1.58	2.36	149.5			
7	23.1	9.0	9.5				1.14	1.43	125.5
7	23.1	9.7	10.0				0.89	1.12	126.0
7	24.2	.....	12.1				0.62	0.84	135.5
7	22.5	8.7	9.7				1.33	1.88	142.0
9	23.4	8.2	9.5				1.15	1.68	146.0

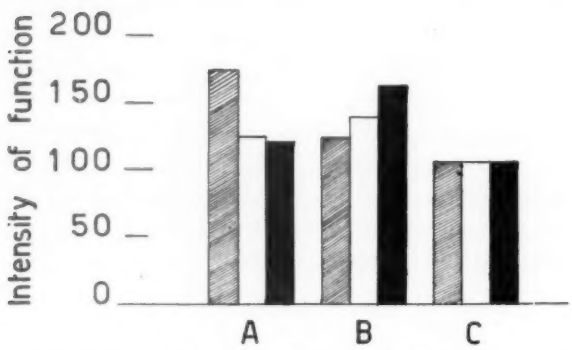

FIG. 2. Relative intensities of photosynthesis (shaded bar), glycolysis (white bar), and respiration (black bar) in wheat plants in a state of defensive reaction (A) to *Oidium*, in a state of receptivity (B) for *Oidium*, and in healthy and normal wheat plants (C). The values for the three metabolic functions in healthy and normal plants have been placed arbitrarily at 100 to give a ratio of 1: 1: 1.

infection, the P-G-R is 1.71 : 1.23 : 1.19 (Fig. 2) (21). In this case, therefore, as in the case of darkness, the attenuation of the defense power of the tissues is characteristically linked with a metabolic balance in which the breakdown processes, especially respiration, predominate over the synthetic process of photosynthesis.[4] A different physiological balance occurs in the tissues of the host, which should be considered responsible for the attenuation of resistance.

Xerophytism and Hygrophytism in Relation to Resistance

Virgilio and Mentana wheat seedlings were grown under xerophytic conditions (much sun and little moisture) on an exposed terrace, and under hygrophytic conditions (little sun and plentiful moisture) in an adjoining greenhouse (27). The ecological differences between the terrace and the greenhouse may be summed up as follows: 1) temperature on the terrace usually a few degrees C. lower, especially at night; 2) relative humidity on the terrace 10–30 per cent lower, particularly during the day; 3) light markedly more intense on the terrace; 4) in the greenhouse, ultraviolet rays cut off by the thick and opaque glass panes.

The seedlings on the terrace were somewhat stunted; the leaves were small, a slightly pale green, always erect. Consequently the seedlings on the terrace never appeared wilted, whereas wilting was general with the seedlings in the greenhouse, especially during the hottest period of the day.

Some pots of the two sets of seedlings were put into a special small greenhouse and heavily infected with *Oidium moniloides;* the seedlings raised on the terrace proved to be very resistant, but the greenhouse plants were severely and rapidly attacked.

[4] This interpretation differs from that suggested in the earlier publication (25); subsequent investigation showed that the present interpretation undoubtedly corresponds better to the series of experiments effected to date.

TABLE 8.—*Photosynthesis in Virgilio and Mentana wheats with hygrophytic and xerophytic habit. The plants were near the earing stage*

Duration of experiment, hours	Temperature (°C.) during the experiment	Mgm. CO_2 fixed by 1 gm. dry matter in 10 liters air		CO_2 fixation by xerophytic plants if value of 100 is given to hygrophytic plants
		Hygrophytic	Xerophytic	
Virgilio				
6	17–22	12.16	18.90	155.5
6	20–25	7.75	8.32	107.0
5½	25–29	6.65	13.33	200.0
Mentana				
6½	13–18	6.12	7.98	130.0
6	16–27	6.28	8.27	131.0
6½	16–28	5.04	6.53	130.0

TABLE 9.—*Respiration and glycolysis in sixth and seventh leaves, in plants near the earing stage, of Virgilio and Mentana wheats with hygrophytic and xerophytic habit*

Temperature (degrees C.) during the experiment	Q_{O_2}		Respiration of xerophytes if value of 100 is given to hygrophytes	Ratio: fresh weight / dry weight Mean value in per cent	
	Hygrophytic	Xerophytic		Hygrophytic	Xerophytic
Virgilio					
24.9	1.60	1.57	98	27.6	32.5
24.9	1.60	1.71	107	28.6	32.5
27.4	2.06	1.82	88	29.2	32.5
27.4	1.38	1.87	135	29.0	31.6
Mentana					
26.3	2.61	2.28	87	22.0	26.0
26.3	2.94	2.43	83	23.0	27.0
27.0	1.99	2.12	106	23.0	26.0
27.0	1.95	1.84	94	25.5	25.5
26.6	2.31	2.16	93	23.0	26.2

Temperature (degrees C.) during the experiment	$Q^{N}_{CO_2}$		Glycolysis of xerophytes if value of 100 is given to hygrophytes
	Hygrophytic	Xerophytic	
Virgilio			
24.5	0.36	0.31	86.0
24.5	0.23	0.29	126.0
24.9	0.28	0.25	89.0
24.9	0.26	0.26	100.0
Mentana			
24.6	0.51	0.48	94.0
24.6	0.45	0.50	111.0
25.7	0.71	0.66	93.0
25.7	0.61	0.54	88.5
25.0	0.62	0.52	84.0
25.0	0.61	0.60	100.0

The results of the experiments on functional ratios are given in tables 8 and 9. The respiratory and glycolytic intensity were practically the same in the two different environments, while the photosynthetic activity appeared to be much stronger in the plants grown on the terrace (particularly the Virgilio variety), despite the lighter color of the leaves.

The mean P-G-R ratio of Virgilio plants grown on the terrace, compared with those raised in the greenhouse, was 1.5 : 1 : 1.1; the ratio for Mentana, 1.20 : 0.95 : 0.97.

This metabolic trend is very similar to that found in the wheat plants during the early days of Oidium infection (21). The physiological physiognomy of the tissues which present the better resistance to this infection, therefore, appears to be markedly constant; it is characterized by the more or less marked predominance of the synthetic processes over the breakdown processes.

Addition of Cadmium to the Nutrient

Resistance may be induced artificially by the addition of cadmium nitrate M/8000 to the nutrient from which the wheat roots may absorb the chemical (8, 10, 15).

TABLE 10.—*Glycolysis of leaves of Virgilio and Frassineto wheats treated with cadmium*

Treatment[a] and its duration	Temperature (°C) during the experiment	Ratio: fresh weight / dry weight Mean value in per cent		$Q^{N}_{CO_2}$	
		Virgilio	Frassineto	Virgilio	Frassineto
First leaf					
Cadmium, 17 days	23.7	12.5	12.9	0.69	0.81
Control	23.7	12.7	12.9	0.81	0.75
Cadmium, 19 days	23.6	11.7	11.7	1.04	1.28
Control	23.6	11.8	11.6	0.90	0.77
Cadmium, 21 days	23.6	12.0	11.8	0.78	0.89
Control	23.6	11.7	11.6	0.85	0.71
Second leaf					
Cadmium, 17 days	24.0	11.5	12.3	0.98	1.50
Control	24.0	12.3	11.5	1.00	1.46
Cadmium, 20 days	25.0	10.3	11.2	0.60	0.70
Control	25.0	12.0	10.2	0.72	0.45
Cadmium, 21 days	24.0	14.5	.....	0.76	.....
Control	24.0	11.3	.....	0.93	.....
Third leaf					
Cadmium, 19 days	25.5	.....	13.0	.....	1.03
Control	25.5	.....	11.6	.....	0.99
Cadmium, 21 days	25.4	13.3	13.3	1.63	1.53
Control	25.4	11.4	12.3	1.32	1.18
Cadmium, 29 days	24.0	.....	13.9	.....	0.77
Control	24.0	.....	10.8	.....	0.58

[a] Not all determinations were made at the same time. Some data were obtained in 1940, some in 1941.

The effect of cadmium varies greatly according to the seven varieties of wheat used (Frassineto, Virgilio, Roma, Rieti, Gentilrosso, Littorio, Mentana). Protection was maximum in Frassineto and minimum in Virgilio; consequently, only these two varieties were taken into consideration, those which had given intermediate results being disregarded. The addition of cadmium nitrate (M/8000) to the nutrient solution for 20 days prior to inoculation with *Oidium* reduced the severity of attack to a value from 1 to 1.5 in Frassineto and to a value from 3 to 3.5 in Virgilio, while the severity values for both control plants were from 4 to 4.5. In spite of the disease differences with cadmium, ash analyses for cadmium in the experimental plants indicated that slightly more cadmium was deposited in Virgilio than in Frassineto. Quantities as high as two parts in 100,000 in the fresh tissues were not markedly toxic to the fungus (15), and therefore it is supposed that cadmium acts indirectly by stimulating the natural metabolic defense power of the host. This interpretation is supported by

TABLE 11.—*Respiration of leaves of Virgilio and Frassineto wheats treated with cadmium*

Treatment[a] and its duration	Temperature (°C) during the experiment	Ratio: fresh weight / dry weight Mean value in per cent		Q_{O_2}	
		Virgilio	Frassineto	Virgilio	Frassineto
First leaf					
Cadmium, 10 days	24.8	11.6	.....	1.66	.....
Control	24.8	11.3	.....	1.58	.....
Cadmium, 11 days	24.1	.....	10.9	.....	1.34
Control	24.1	.....	11.6	.....	1.44
Cadmium, 12 days	23.6	10.2	.....	1.60	.....
Control	23.6	9.7	.....	1.39	.....
Cadmium, 15 days	24.4	11.7	13.4	1.43	1.54
Control	24.4	12.2	12.0	1.33	1.47
Cadmium, 16 days	24.0	12.5	12.8	2.14	1.72
Control	24.0	13.1	13.1	1.66	1.58
Second leaf					
Cadmium, 11 days	24.1	.....	13.3	.....	1.41
Control	24.1	.....	12.7	.....	1.33
Cadmium, 12 days	23.6	12.2	.....	1.33	.....
Control	23.6	11.2	.....	1.20	.....
Cadmium, 20 days	25.2	14.4	14.0	1.26	1.33
Control	25.2	11.8	11.8	1.25	1.41
Third leaf					
Cadmium, 20 days	25.4	11.9	12.8	1.14	1.11
Control	25.4	10.2	10.5	1.15	1.19
Cadmium, 22 days	25.3	.....	11.9	.....	1.48
Control	25.3	.....	9.8	.....	1.38
Cadmium, 24 days	26.0	11.8	11.7	0.92	1.20
Control	26.0	9.6	9.3	0.92	1.42
Cadmium, 28 days	25.2	.....	14.1	.....	1.34
Control	25.2	.....	11.7	.....	1.49

[a] Not all determinations were made at the same time. Some data were obtained in 1939, some in 1940.

the fact that Frassineto, although absorbing slightly less cadmium than Virgilio, has a much stronger defense power against *Oidium*.

With cadmium treatment, too, the glycolytic power increased to a greater and more constant extent in Frassineto than in Virgilio (Table 10). As regards the respiratory power, a very slight stimulation was noted in Virgilio, but it did not seem to be affected in Frassineto (Table 11).

The mean value of the G-R ratio was 1.28 : 0.98 for Frassineto and 0.97 :

FIG. 3. Refracting granules (G) near nucleus (N) in epidermal cells of Frassineto wheat 52 days after treatment with cadmium nitrate and 18 days after inoculation with *Oidium* (×447).

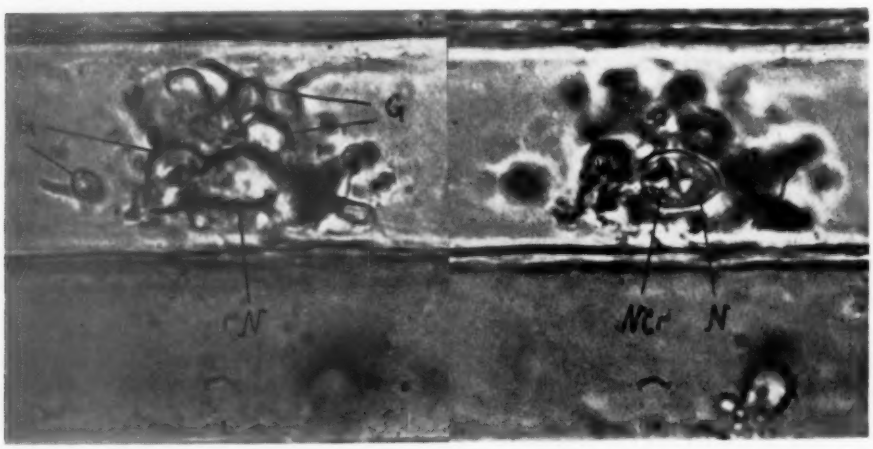

FIG. 4. Left: Granules (G) covering the nucleus (N) in a cell of Virgilio wheat exposed to continuous light for 10 days and returned to normal light for 5 days. Photographed 15 days after inoculation with *Oidium* (×715). Right: The same cell photographed at a different focus level to bring out the nucleus (N) and nucleolus (NC). The granules are not in focus (×715).

1.08 for Virgilio. Therefore, the ratio changed in favor of glycolysis only in Frassineto, the variety best protected by cadmium.

At present we have not sufficient evidence for conclusions regarding changes in photosynthesis that follow treatment with cadmium.

Refracting granules frequently appear in the epidermal cells of wheat seedlings treated with cadmium nitrate (especially the Frassineto variety) or exposed to continuous light (10). The granules usually form close to the nucleus and are resistant to ordinary staining reagents (Figs. 3 and 4). It is believed that the granules are of a lipoprotein nature and that they indicate the formation and accumulation of insoluble compounds.

UTILIZATION OF ISOMERS

No direct data are available as regards the isomeric variations within the tissues of the host, but the following observations, obtained mainly from studies on bean rust, are presented. The primary leaves of beans were infected with urediospores of *Uromyces appendiculatus;* 3 days later, when the mycelium was spreading through the mesophyll, the leaves were cut and floated on sugar solutions in crucibles. Different isomers were examined, selected from among the ketohexoses (*d*-levulose, *d*-sorbose), aldohexoses (*d*-glucose, *d*-mannose, *d*-galactose), pentoses (*l*-xylose, *l*-arabinose), and disaccharides (saccharose, maltose, lactose), all in 1 per cent solution. A crucible containing distilled water served as control. All the crucibles were placed in the dark to prevent photosynthesis and thus oblige the leaves to utilize, for themselves and for the parasite, the sugar at their disposal. Every 2 to 3 days the solutions were changed to avoid fermentation; after 9 to 10 days the intensity of disease attack on the leaves was recorded.

In distilled water the leaves had yellowed and were partially necrotic, without any trace of the disease; evidently the deterioration was due to glucidic denutrition, and consequently the parasite had not been able to develop. In levulose the leaves appeared green and in fairly good condition, but were covered to a large extent by the uredosori of the parasite; the levulose, therefore, was easily utilized and assimilated by both the leaf and the parasite. With sorbose, the leaves were practically normal green, in fairly good condition, and had only traces of uredosori, nearly all of which were aborted; only the bean leaves had been able to utilize this sugar (levulose isomer), while the parasite had been incapable of using it for its own metabolism (13).

The experiment was repeated several times with identical results.

If it is considered that these two sugars differ from each other only by the position of an OH in the molecule, it will easily be understood how important it would be for the plant, in regard to resistance to a specific parasite, to be able eventually to change, under the stimulus of parasitism, its metabolism from a series of isomers easily utilized to one less easily or not utilized by the pathogen.

Although there is no direct proof of the capacity of the plants to carry

out, in their own nutritional interests, this isomeric deviation under parasite stimulation, nevertheless the experiments effected permit foreseeing as possible and probable a resistance of this kind, which could be very effective though remaining within the purely physiological field. This also would be a strictly metabolic resistance, with the formation of compounds not poisonous for the parasite nor for the plant, but utilizable only by the latter, while the parasite would die of inanition.

DISCUSSION

The plants studied, and in particular wheat, presented a different physiological physiognomy according to whether they were in a state of receptivity or resistance to their parasites.

It is the ratio of intensity between the principal functions, the functional balance, which varies.

The investigation on the metabolism of the "wheat-*Erysiphe* complex," conducted systematically over the entire cycle of the disease, revealed, during the first phase of parasitism, a markedly increased accentuation in the synthetic processes, compared with the breakdown processes. In the final phase of the disease, however, the physiological physiognomy was completely reversed, and the breakdown processes, linked with the respiratory activity, attained very high values, while the synthetic processes (linked with photosynthesis) were much below normal. The P-G-R ratio was 1.71 : 1.23 : 1.19 in the initial phase of parasitism, and 0.45 : 0.56 : 3.82 in the final phase.

The P-R ratio, considerably over 1 in the initial phase of the disease, tended to diminish rapidly during the successive stages of infection, until, in the last stage, the values were not much over 0. At the onset of the disease the photosynthetic intensity, therefore, was almost double the respiratory intensity, compared with normal; at the end of the disease cycle, the former was 1/8–1/10 of the latter.

Similar investigations are being carried out on other "complexes" (bean– *Uromyces;* lettuce– *Bremia;* wheat– *Puccinia*); in the main, the results obtained to date appear to confirm the findings for the "wheat-*Erysiphe* complex."

The abnormal increase in photosynthesis which, under normal light conditions, occurs during the first days of parasite attack should be considered as a defensive reaction; in fact, if photosynthesis is checked by placing the plants in the dark during the early period of infection, attack is always appreciably more severe than usual. In addition, on measuring the metabolism of healthy plants kept in the dark for a few days, it was found that darkness also modifies substantially the glycolysis-respiration ratio. In the obscured plants the G-R was 0.45 : 0.70 for wheat and 0.32 : 0.70 for lettuce. Therefore, during darkness, considering the photosynthesis as being checked, the P-G-R was approximately 0 : 45 : 0.70. In daylight the defensive reaction (during the early days of infection) resulted in a ratio of about 1.71 : 1.23 : 1.19. It may be concluded that darkness causes

a marked disequilibrium in the functional ratios, leading to a decided predominance of the respiratory process, due not to a stimulus of respiration but to a very considerable depression of the other two functions. As regards photosynthesis, it should be noted that not only its intensity (compared with the other two processes), but also its duration is very important. In effect, it was found that wheat exposed to continuous artificial light for several days remained practically immune from Oidium attack.

The enormous functional disequilibrium observed at the end of the disease cycle indicates that the tissues of the plant have by this time lost all control of their metabolism; this very serious disequilibrium of functional balance may be considered as the essence of the disease in that it is the cause of the morphological changes and subsequent death of the host cells (23, 26). The parasite, however, has now reached the final phase of its development, having produced a plentiful supply of conidia.

Other experiments showed that healthy wheat plants grown under conditions of relative xerophytism were resistant to Oidium attack and had a functional balance in which the synthetic processes were prominent. The P-G-R was 1.5 : 1 : 1.1 compared with the plants raised under conditions of relative hygrophytism (ordinary greenhouse). On the other hand, when healthy wheat plants were grown under marked hygrophytic conditions (shaded greenhouse) and wilted through sudden exposure to full sunlight, there was a marked and lasting predominance of the respiratory activity over the other two processes, together with a reduction in turgidity and an increase in receptivity. The P-G-R was found to be 1.23 : 1.43 : 1.60 (compared with the control plants not exposed to sunlight).

As regards wheat, at least, the experimental results appear to be sufficiently clear and are concordant in delineating the physiological physiognomy which characterizes defense and predisposition respectively. On examining the balance between photosynthesis, glycolysis, and respiration, it will be seen that in plants in a state of defense, the synthetic processes generally predominate over the breakdown processes, while the contrary takes place in plants in a state of receptivity. It should be noted, in particular, that when the P-R ratio is over 1 (considering this ratio in the control plants arbitrarily as 1), the tissues of the plants are in a state of metabolic resistance stronger than normal; when, instead, this ratio falls below 1, the resistance decreases proportionately. Very likely this fact is also connected with an enrichment of the substratum in high molecule compounds (sparingly soluble and therefore not very assimilable by the parasite) in opposition to a relative impoverishment in low molecule compounds (soluble and therefore easily utilizable by the parasite).

In addition, being a question of variation in the ratios of intensity between the specifically endothermic processes (synthesis) and the specifically exothermic processes (breakdown), it may be assumed that the energizing disequilibrium which ensues from the variation in these ratios has, in itself, its effect in determining an increase or diminution in defense. This ap-

pears all the more likely since the parasites, being symbiotic, utilize the biochemical reactions of the substratum as a source of both energy and nutrients. The energizing, besides the plastic disequilibrium, therefore, has a far from negligible importance.

If examination is limited to the glycolysis-respiration balance, it is found that generally, in plants in a state of defense, the ratio changes and glycolysis predominates. Thus the seven varieties of wheat exposed to continuous light and the Frassineto variety given preventive treatment with Cd, showed a considerable increase in their resistance to *Oidium*, together with a corresponding, but less marked, increase in the glycolytic power compared with respiration. In the first case (continuous light), G-R = 1.53 : 1.14; in the second (Cd), G-R = 1.28 : 0.98.

The G-R balance is inverted in plants in a state of predisposition. In fact, it was observed that in wheat and lettuce plants predisposed by darkness, the G-R ratio was 0.45 : 0.70 and 0.32 : 0.70, respectively; in wheat plants predisposed by wilting, G-R = 1.43 : 1.60.

The accentuation of the glycolytic power in comparison with the respiratory power, therefore, appears to be a sign of increase in metabolic resistance. The G-R ratio also, like the P-R ratio, tends to assume values over 1 in tissues in a state of defense and values less than 1 in predisposed tissues. It is probable that the accumulation of the products of this fermentative process may, within certain limits, be toxic for the parasite.

The sum of the experimental data and the findings discussed so far logically lead to rejecting, as not corresponding, the conception that metabolic predisposition is linked with a general attenuation of the metabolism of the plant (7), or that the state of metabolic defense depends on a general stimulus of the metabolism itself. In the case of wheat, it was found that both the plants in a state of defense (initial reaction against the spread of the parasite) and those in a state of predisposition (through wilting) had a markedly "stimulated" metabolism compared with normal, but the equilibrium of the functions was inverted (1.71 : 1.23 : 1.19 as against 1.23 : 1.43 : 1.60). Therefore, only the functional ratios change. From this point of view we could also consider as protected a tissue with metabolism generally attenuated, compared with normal, provided that there is a definite predominance either of photosynthesis or of glycolysis (for example, P-G-R = 0.7 : 0.5 : 0.3); such cases, however, have not yet occurred under our experimental control.

For the moment we can only confirm that an unbalanced increase in the respiratory rate was always found to be linked with a more or less marked diminution in the metabolic defense, while an unbalanced stimulus in the photosynthetic or glycolytic intensity always appeared to be closely correlated with an increase in the defensive tone of the tissues.

Consequently, all ecological conditions or treatments which stimulate the respiratory activity in disproportion to the other functions produce a state of predisposition in the tissues. A special state of metabolic defense

arises, however, when the ecological conditions or treatments stimulate only, or preferably, the photosynthetic or glycolytic activity over the respiratory process.

It has been proved, therefore, that firstly, there is actually a well-defined type of metabolic resistance linked with the rate of anabolism and catabolism of the host plant, and that the efficacy of this defense should not be underestimated; secondly, that the environmental conditions may have a decided influence on the physiological balance, causing an increase or diminution in this protection mechanism; thirdly, that it is possible by special means (administration of various substances in nontoxic doses; treatment with continuous light; sudden insolation, etc.) to modify this defense positively or negatively.

One more point: We have seen functional disequilibrium occur in (a) tissues which modify positively or negatively their metabolic defense compared with normal; (b) tissues consumed by the morbid process and especially when the final stage approaches. The intensity and behavior of this disequilibrium, however, is very different. The disequilibriums relative to the accentuation or diminution of the state of metabolic defense are comprised within rather modest, we might say, physiological limits; those regarding the pathological state, particularly in the final stage of the disease, instead, are extraordinarily accentuated, and justify the interpretation that they themselves constitute the essence of the disease (23).

It is emphasized, at least as regards disease of parasitic origin, that, except for the different proportions, the same physiological physiognomy characterizes both the plants in a state of predisposition and the plants in an advanced morbid stage: the predominance of the respiratory process over the photosynthetic and glycolytic processes. This explains how predisposition may, in a sense, be considered as an initial stage of the disease.

As regards the possibility of isomeric transposition in the tissues of the host plant following parasitic attack, the experiments have not yet been sufficiently exhaustive to permit giving an affirmative opinion; and we are still less certain whether it is possible, with physical or chemical treatments, to stimulate the plant towards this type of isomeric defense, a type which is within the purely physiological range, harmless for the host, and yet extremely effective. We know, however, that by making the plant insert in its metabolism a glucide not easily utilized by the parasite in place of one which it easily assimilates, the morbid process can be rapidly and almost completely checked.

These investigations, which are still in the preliminary stage although they have been carried out over a period of ten years at the Institute of Plant Physiopathology, University of Perugia, are of only general and theoretical interest for the present; it is to be hoped, however, that taken up by other researchers, and with more ample means, this work may lead to practical applications not only in the phytopathological field, but also in the agricultural and genetic domain.

SUMMARY

The conception of "metabolic resistance" is explained, and the mechanisms through which it appears to operate are indicated.

The metabolism of the host-parasite complex is outlined and the defensive nature of the metabolic reactions which appear at the onset of parasitism is discussed; the physiological reasons for the final collapse are also explained.

The experimental data show the importance of the ratio of intensity between photosynthesis, glycolysis, and respiration, in determining the establishment of a state of metabolic resistance or of metabolic receptivity of the host tissues.

Ecological conditions have a decisive influence in determining changes in functional balance of the host plant and, therefore, its state of resistance.

Details are given on the various systems for intervening artificially, by physical or chemical treatments, in the defensive state of the plant, by changing its functional balance.

Lastly, the experimental data give evidence of the importance of the possibility, for the defensive mechanism of the plant, of stimulating isomeric transposition in the compounds preferred by the parasite, during the anabolic and catabolic phases of the living metabolism.

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STUDIES ON THE PARASITISM OF RHIZOCTONIA SOLANI KUEHN ON SOYBEANS[1]

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Field surveys have indicated that root rot caused by *Rhizoctonia solani* Kuehn is potentially a destructive disease of soybeans in Minnesota. In the present work, 16 isolates of *Rhizoctonia solani* from various legume, cereal, field crop, and vegetable hosts (Table 1) were studied in culture and were

TABLE 1.—*Source of isolates of Rhizoctonia solani used for experiments*

Isolate number	Host	Locality	Year of isolation
1	Sweet clover	Manitoba, Canada	1934
2	Soybean root	Iowa	1946
3	Soybean root	University Farm, St. Paul, Minnesota	1946
4	do	do	1946
5	do	Iowa	1946
6	do	University Farm, St. Paul, Minnesota	1946
7	Barley root	do	1946
8	Muskmelon	do	1946
9	Flax	Unknown	1937
10	Sugar beet	Chaska, Minnesota	1930
11	Tomato	Unknown	1946
12	do	do	1946
13	do	do	1946
14	Flax root	Red Wing, Minnesota	1937
15	Tomato	Unknown	1946
18	do	do	1946

tested for their virulence on soybeans. Filtrates secured from certain of the isolates grown in culture were tested for their effects on soybeans.

Peltier (10), who was the first to study extensively the parasitism of *Rhizoctonia solani*, found no outstanding differences in the pathogenicity of a number of isolates. Subsequently, however, other workers (3, 4, 5, 6, 7, 8, 12, 13) demonstrated that isolates may differ greatly in cultural characters and pathogenicity.

VIRULENCE OF ISOLATES ON SOYBEANS

Greenhouse and field experiments were made to determine whether cultural races of *Rhizoctonia solani* differed in pathogenicity on soybeans.

[1] This work was done under the guidance of Dr. Ian Tervet, formerly Assistant Professor of Plant Pathology, University of Minnesota, and Collaborator, Division of Forage Crops and Diseases, U. S. Department of Agriculture, and Dr. M. F. Kernkamp, Assistant Professor of Plant Pathology, University of Minnesota, and Collaborator, Division of Forage Crops and Diseases, U. S. Department of Agriculture, in partial fulfillment of the requirements for the Master of Science degree. Paper No. 2461 of the Scientific Journal Series of the Minnesota Agricultural Experiment Station.

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Inoculum was increased on a soil medium consisting of 1 part loam soil, 5 parts sand, and cornmeal equivalent to 3 per cent of the total weight of the soil mixture. The methods and materials used to infest soil were similar to those used by Storey (12). Inoculum was applied at the rate of 5 gm. to 160 gm. of air-dried soil, equivalent to about 95 gm. of inoculum for each 4-in. pot and 200 gm. of inoculum for each 6-ft. field row.

In the winter of 1946–1947, 15 of the 16 isolates were tested for virulence on four soybean varieties in a greenhouse maintained at an average temperature of 27° C. Ten healthy soybean seeds of one variety were planted in each 4-in. pot, and four pots, one of each variety, constituted a unit for replication (Table 2). Each treatment was replicated four times. Final

TABLE 2.—*Average stands for five varieties of soybean in soil infested with isolates of Rhizoctonia solani. Soil infested 2 days prior to planting, emergence recorded 20 days after planting in greenhouse or 14 days after planting in field*

Isolates of *Rhizoctonia solani*	Average number of seedlings emerged[a]					
	Greenhouse tests				Field tests	
	Habaro	Bansei	Minsoy	Manchu Wis. No. 606	Minsoy	Manchu Wis. No. 3
1	5	4	6	2	12	15
4	6	2	4	5	10	9
5	6	5	2	10	16	17
9	17	14	15	18	.....	.....
2	24	16	20	21	.....	.....
14	24	20	22	21	28	27
3	17	20	25	25	40	37
8	23	20	25	22	.....	.....
13	24	27	23	22	.....	.....
6	22	26	22	27	37	39
12	31	30	27	30	.....	.....
18	.....	.....	.....	.....	39	36
10	34	29	30	32	40	37
11	27	34	32	33	45	41
15	35	30	37	30	.....	.....
7	32	34	30	29	.....	.....
Control	40	39	40	40	42	40
L. S. D.	13.8	13.8	13.8	13.8	5.1	6.5

[a] The averages for greenhouse tests represent the average number of plants that emerged in each of the four replicates. Perfect emergence for each replicate would be 40. The average for the field tests represents the average number of plants that emerged in 6-ft. rows in which perfect emergence would be 50.

readings on stand and lesions occurring on stems and roots were taken 20 days after planting, and the isolates of Rhizoctonia originally used to infest the soil were isolated from lesions on the plants.

Field experiments were made in the fall of 1946 and in the spring of 1947 to determine the pathogenicity of nine isolates of Rhizoctonia on two varieties of soybean; these results also are in table 2. The variety Minsoy was planted August 27, 1946; the variety Manchu Wisconsin No. 3, May 17, 1947.

The field plan for both fall and spring experiments was a simple, random-

ized block pattern. Each treatment was replicated four times. The rows were 6 ft. long, and the distance between rows and blocks was 3 ft. Fifty seeds were planted about 2 in. deep in each row. Seedling emergence was recorded 14 days after planting, when each seedling, with its taproot intact, was removed from the soil, rinsed in water, and examined for stem and root lesions.

According to analysis of variance of greenhouse data on stand, the variance between isolates of Rhizoctonia was significant, whereas that between the four soybean varieties was not. Isolates 1, 4, and 5 caused, on the average, 80 to 90 per cent damping-off of the soybean varieties Habaro (Minn. Acc. No. 109), Bansei, Minsoy (Minn. Acc. No. 139), and Manchu Wisconsin No. 606 (Minn. Acc. No. 215) (Table 2 and Fig. 1). Isolates 2,

FIG. 1. Emergence of Habaro soybean seedlings 20 days after 10 seeds were planted in each pot of soil infested with an isolate of Rhizoctonia. A through H, isolates 1, 4, 5, 8, 6, 10, 15, and 7, respectively. I, control.

3, 6, 8, 13, and 14 damped-off about 50 per cent of the seedlings; isolates 7, 10, 11, and 15, about 15 per cent.

The pathogenicity of the 16 isolates on the four soybean varieties was

somewhat generalized in that an isolate strongly pathogenic on one soybean variety was also strongly pathogenic on the other three varieties, and an isolate weakly pathogenic on one variety was also weakly pathogenic on the other three. The vegetable variety Bansei, however, was somewhat more susceptible to damping-off than the other three varieties.

Analysis of variance on field data also shows that the difference between isolates of Rhizoctonia was statistically significant. Isolates 1, 4 and 5 caused about 85 per cent damping-off in the varieties Manchu Wisconsin No. 3 and Minsoy. Isolate 14 was moderately pathogenic; isolates 3, 6, 10, 11, and 18 were weakly pathogenic. In general, the field results with isolates 1, 4, 5, 10, and 14 on Minsoy agreed with those obtained in the greenhouse; isolates 3, 6, and 11 were somewhat less pathogenic than they had been in the greenhouse (Table 2).

To record the severity of disease on stems and roots of infected soybean seedlings five arbitrary classes of lesion types were established: Class 0—Healthy seedlings free of stem and root lesions. Class 1—A few brown, superficial lesions on epidermis of either stem or root. Class 2—Numerous but superficial lesions extending not more than three cell layers into the cortex of stem or root. Class 3—Numerous and large lesions extending deep into the cell layers of the cortex of stem or root. Class 4—Large necrotic lesions extending beyond the cortex into the vascular stele and often girdling stem or root.

TABLE 3.—*Disease index produced by isolates of Rhizoctonia on soybean varieties grown in greenhouse and field*

Isolates of *Rhizoctonia solani*	Index based on lesion class[a]					
	Greenhouse tests				Field tests	
	Habaro	Bansei	Minsoy	Manchu Wis. No. 606	Minsoy	Manchu Wis. No. 3
5	4.0	4.0	3.8	3.7	3.9	3.8
4	4.0	3.6	4.0	4.0	3.8	3.5
1	3.7	4.0	3.6	3.7	3.6	3.5
2	2.3	2.5	2.0	2.2	.....	.....
14	1.0	1.4	1.6	1.8	3.0	2.5
6	1.6	1.8	1.8	1.0	1.0	1.3
3	0.0	0.4	0.0	0.0	1.8	1.3
9	0.8	0.8	0.9	1.0	.....	.....
7	0.6	0.7	0.4	0.4	.....	0.8
8	0.4	0.5	0.3	0.2	.....	.....
11	0.2	0.4	0.0	0.3	0.2	0.4
10	0.0	0.2	0.0	0.0	0.8	0.0
13	0.0	0.4	0.0	0.0	.....	.....
18	0.0	0.3	0.0	0.0	.....	.....
12	0.0	0.3	0.0	0.0	.....	.....
15	0.0	0.0	0.0	0.0	0.0	0.0
Control	0.0	0.0	0.0	0.0	0.0	0.0

[a] Key to lesion classes: 0, no lesions present on stems or roots; 1, superficial lesion not involving the epidermal layer; 2, lesions involving one to three cortical cell layers; 3, lesions involving three to six cortical cell layers; 4, lesions involving vascular elements and often girdling stem or root.

A disease index was calculated according to the method used by Ortega (9). To determine the classes, all the plants of one variety having the same lesion type were grouped together. The disease index for each isolate on a single soybean variety was calculated by multiplying the number of plants in each class by the class value (0, 1, 2, 3, or 4), adding the products, and dividing by the total number of plants observed. For example, the formula $\frac{(30\times4)+(10\times3)}{40}$ gave a disease index of 3.7 for isolate 1 on the varieties Habaro and Manchu Wisconsin 606 in the greenhouse test (Table 3). Thirty seedlings had lesions of class 4, and 10 seedlings had lesions of class 3.

Isolates 1, 4, and 5 produced girdling lesions (class 4) on the stems and roots of the soybean varieties grown in the greenhouse and field (Table 3 and Fig. 2). The disease indexes produced by the other isolates in the greenhouse and field (Table 3) ranged from 0.0 to 3.0.

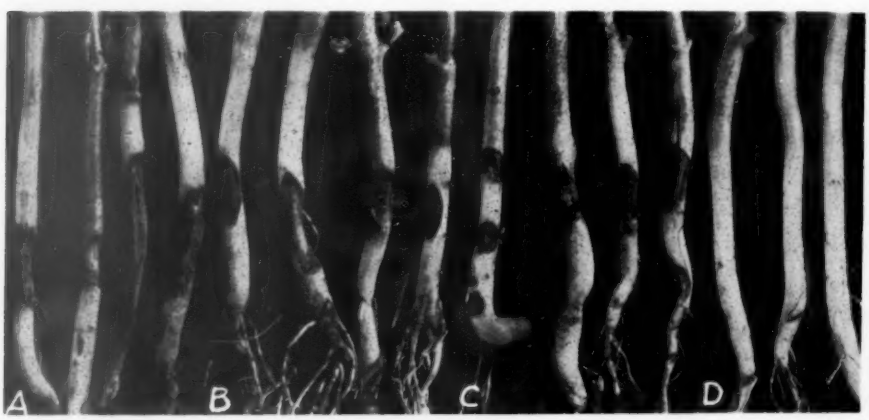

FIG. 2. Manchu Wis. No. 3 soybean seedlings with class 4 necrotic lesions produced by Rhizoctonia: A, isolate 5; B, isolate 4; C, isolate 1; D, control.

Of eight isolates tested both in the greenhouse and in the field on five soybean varieties, isolates 1, 4, and 5, originating from sweet clover and soybeans, were the most virulent on the basis of damping-off and disease index; isolate 14, originating from flax, was moderately pathogenic. Isolates 3 and 6 were moderately pathogenic in causing damping-off in the greenhouse and weakly pathogenic in the field; isolate 6 also had a high disease index while isolate 3 had a low disease index. In regard to both damping-off and disease index, isolates 10 and 11 were weakly pathogenic in the greenhouse and field. Of seven isolates tested only in the greenhouse on four soybean varieties, isolates 2, 8, 9, and 13 were moderately pathogenic in causing damping-off but only isolate 2 had a fairly high disease index. Isolates 7, 12, and 15 were weakly pathogenic in causing damping-off and also had a low disease index.

Virulence of Two Isolates at Various Soil Temperatures

The strongly pathogenic isolate 1 and the moderately pathogenic isolate 14 again were tested for their virulence on Habaro soybeans grown in soil

temperature tanks controlled at 14°, 19°, 21°, 25°, 29°, and 33° C. Neither isolate caused appreciable damping-off at 14° C. At 19° and 21° C., isolate 1 reduced the stand by 15 per cent and isolate 14 by 10 per cent. The most damping-off occurred at the optimum temperatures of 25° and 29° C.—100 per cent with isolate 1 and 95 per cent with isolate 14. At the highest temperature (33° C.) both isolates reduced the stand 60–70 per cent. Besides difference in pathogenicity, the isolates differed in cultural characters when grown on four different media.

CULTURAL CHARACTERS OF ISOLATES

Six isolates of *Rhizoctonia solani* from five crops (Table 1) were used in these studies. Three (1, 4, and 5) were strongly pathogenic, one (8) was moderately pathogenic, and two (7 and 15) were weakly pathogenic on soybeans (Table 2). The isolates were grown in Petri plates on potato agar, malt agar, soybean agar, and Smith-Humfeld agar. Each isolate was replicated four times on each of the four media. They were incubated for 13 days at 26° C. before notes on cultural characters were taken.

The six cultural isolates on the four media differed on the basis of the general characters of the colony and sclerotia. The color of the colonies varied from drab to various shades of brown. Color of sclerotia differed for races 1, 8, and 15, but was similar for races 5 and 7; no sclerotia were produced by isolate 4. Aerial mycelia were produced abundantly by isolates 1 and 4, and sparsely by isolates 5, 7, and 8; none were produced by isolate 15.

The cultural characters of the six isolates were not correlated with pathogenicity. For example, sclerotia are formed both by the weakly pathogenic isolate 15 and by the strongly pathogenic isolate 5. A lack of correlation between cultural characters and pathogenicity was also noted for other characters, namely, zonation, abundance of aerial mycelium, color of the colony, furrowing, color, size, and abundance of the sclerotia.

The difference in pathogenicity and cultural characters between the isolates constitutes a basis for calling them physiologic races of *Rhizoctonia solani*.

PENETRATION OF CELLS OF SOYBEAN SEEDLINGS BY RHIZOCTONIA SOLANI

The mode of hyphal penetration in soybean seedlings was studied, both in unstained, freehand sections of fresh roots and stems and stained sections of tissue embedded in paraffin. The freehand, unstained sections were more satisfactory than the embedded material, because cell shrinkage was negligible. Moreover, the brown discoloration of the cell walls and protoplasm in advance of hyphal penetration were very distinct in the freehand sections but could not be seen in stained sections.

Minsoy seedlings were grown in sterilized vermiculite inoculated with one of the three races of Rhizoctonia, 1, 4, or 6, 6 days before planting the

seed. The average room temperature during the course of the experiment was 26° C. The seedlings were watered with sterile distilled water.

About 150 longitudinal and cross sections of necrotic tissues on seedlings were cut freehand from infected seedling stems and taproots, mounted in

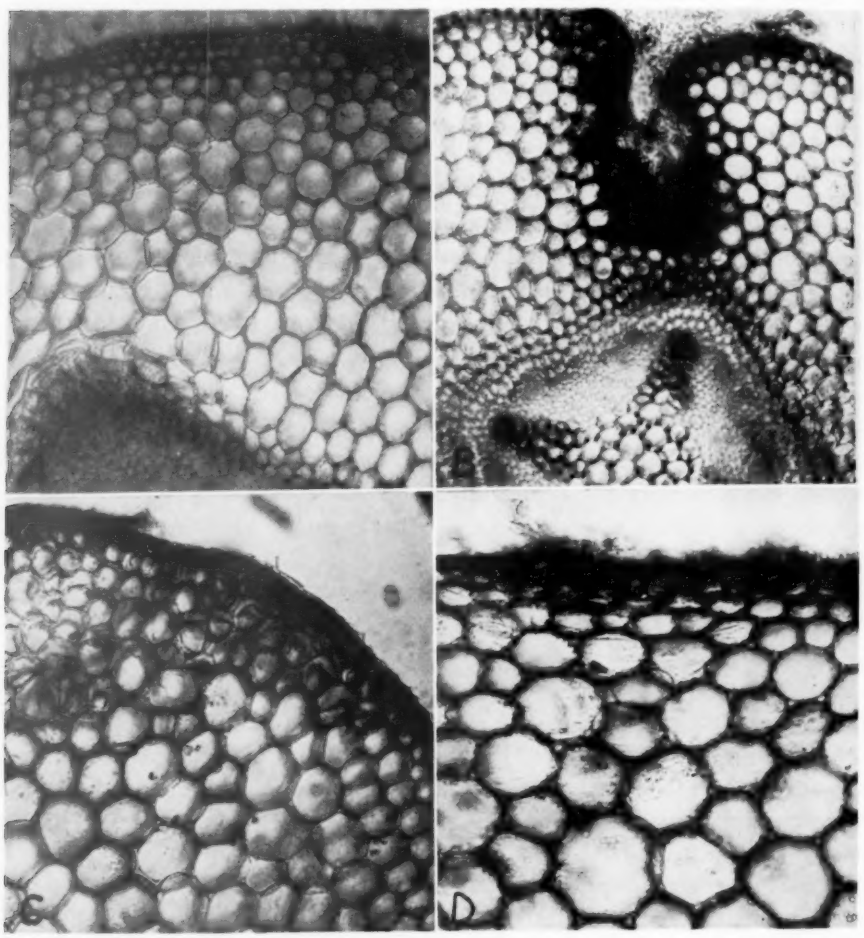

FIG. 3. Freehand, unstained cross sections of soybean taproots. A. Healthy root. B. Rhizoctonia isolate 4 infecting a cracked taproot. Walls and protoplasm are discolored 2–6 layers of host cells bordering the crack. C. Rhizoctonia isolate 1 infecting taproot. Walls discolored 1–8 layers of host cells in advance of the penetrating hyphae. D. Taproot naturally infected with *Rhizoctonia solani* in the field. Walls discolored 2–12 layers of host cells in advance of the penetrating hyphae. Magnification of A–C, 40×; D, 50×.

Patterson's mounting medium[3] and examined microscopically. Penetration by peglike hyphae, as reported by Matsumoto (8), Abdell Salam (1), and Tsiang (13), could not be seen in any of the sections examined. In all sections, however, there appeared a definite, dark brown discoloration of the cell walls and often of the cell protoplasm in advance of the penetrating

[3] Patterson's mounting medium: potassium acetate (2 per cent in water), 50 cc.; glycerine, 20 cc.; alcohol (95 per cent), 30 cc.

hyphae. The discolored area usually extended laterally 2–6 cells beyond the limits of the lesion and 2–10 cell layers deep (Fig. 3, B–D). The discoloration of the cell walls and protoplasm was also observed in the sections of necrotic lesions occurring on the cracked side of the taproot. These natural cracks are frequent on taproots of seedlings and may be a good avenue of entrance for *Rhizoctonia solani* hyphae. The hyphae can be seen within the cracks and the brownish-black discolored area can be observed in advance of the penetrating hyphae (Fig. 3, B). In all cases the host tissue was discolored before being invaded by hyphae.

Infected portions of seedling stems and taproots were killed and fixed in chromic acid, acetic acid, and formaldehyde solution (CRAF). The dehydration, infiltration, and embedding procedure followed was that described by Sass (11). Cross and longitudinal sections were cut 12, 16, and 18 μ, and stained with Flemming's triple stain (11). The exact mode of hyphal penetration could not be determined from either the paraffin or freehand sections.

THE EFFECT ON THE HOST OF FILTRATES OF THE FUNGUS

Ortega (9) demonstrated that the filtrates of some isolates of Rhizoctonia inhibited secondary root development and caused wilting of tomatoes and beans. Tsiang (13) also found that the filtrates of isolate 9 caused wilting of week-old flax seedlings.

As stated above, the host cells were definitely discolored before being invaded by hyphae. When typical Rhizoctonia lesions on stems and roots of soybean seedlings grown in the field were examined, many of them appeared to be free of hyphae. It apeared, therefore, that diffusible substances from Rhizoctonia hyphae might have caused the symptoms. Accordingly, experiments were made to test the hypothesis.

In the experiments on toxicity, races 1 and 4 had been strongly pathogenic in the field and greenhouse, causing severe damping-off and pronounced necrotic lesions on stems and roots; race 6, on the other hand, was only weakly pathogenic.

These three races were grown in Petri plates, each of which contained 20 ml. of potato-dextrose agar taken from a single batch of medium. When the cultures were about 10 days old, plugs of inoculum were cut from the edges of the colonies and transferred to 250-ml. Erlenmeyer flasks containing 80 ml. of one of the following nutrient solutions: Richards' solution, peptone broth, and potato-dextrose broth. Controls received plugs of non-inoculated potato-dextrose agar. The cultures grown in peptone were incubated 15 days and the cultures in potato-dextrose broth and Richards' solution were incubated 30 days.

At the end of the incubation period the liquid solution was filtered from the mycelial mat. The solution was then passed through a sterile Seitz filter and collected in sterile test tubes in 20-ml. portions. The control solution was similarly filtered and collected. The filtrates from peptone and

Richards' solution were then transferred to sterilized Erlenmeyer flasks which contained a thin layer of cotton. Ten ml. of sterile distilled water were added to the 20 ml. of the filtrate in each flask. Portions of the filtrates from isolates 1, 4, and 6 were autoclaved for 15 min. at 15 lb. pressure (250° F.). These stock filtrates were tested for their effects on seed germination and growth of young soybean plants.

Seed Germination

Ten surface-disinfected seeds of Minsoy were planted in each of four flasks and incubated for 20 days at 26° C.

The autoclaved and nonautoclaved filtrates from the strongly pathogenic races 1 and 4 reduced seed germination about 45 per cent and inhibited development of secondary roots, consequently stunting the growth of the plants (Fig. 4, A and B). The autoclaved and nonautoclaved filtrate from the weak race 6 did not significantly reduce seed germination or inhibit root development (Fig. 4, C).

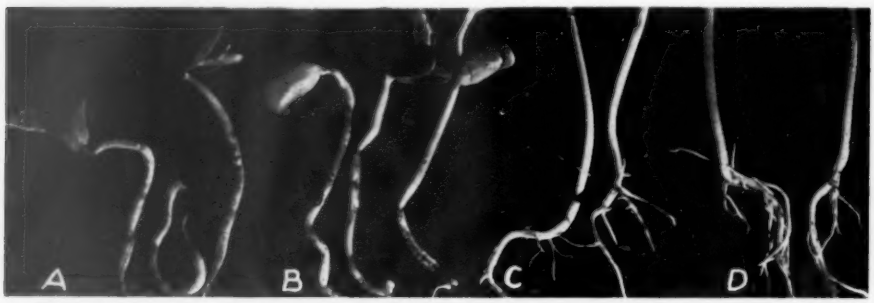

FIG. 4. Seedling development of Minsoy treated with filtrates from two races of *Rhizoctonia solani* grown for 15 days on peptone broth. A. Seeds germinated in filtrates from race 1. B. Same as A except that filtrates were sterilized. C. Seeds germinated in filtrates of race 6. D. Seeds germinated in the noninoculated (control) solution.

Composition of the medium and time of incubation affected the toxicity of the filtrates. The filtrates of races 1 and 4 grown on potato-dextrose broth were not toxic to soybean seeds or seedlings even when the cultures were incubated for 30 days. Cultures of races 1 and 4 in peptone nutrient broth did not yield toxic filtrates unless they had been incubated for at least 15 days. And in Richards' solution only the filtrates obtained from cultures incubated for 25–30 days were toxic.

Development of Seedlings

Minsoy soybean seeds were surface-disinfected in 1:1000 mercuric chloride for 3 min., rinsed in 1 per cent sodium hypochlorite for 1 min., and then placed aseptically on moistened filter paper in sterile Petri plates. Five days after germination each seedling was transferred to a 250-ml. Erlenmeyer flask containing 30 ml. of autoclaved and nonautoclaved stock filtrate from the same batch used for the previous experiment. The seedlings in the filtrate were incubated for 15 days at 26° C.

The filtrate from the strongly pathogenic race 1 inhibited development of secondary roots and, consequently, the seedlings were stunted (Fig. 5, A

FIG. 5. Development of 5-day-old Minsoy seedlings treated with filtrates from two races of *Rhizoctonia solani* grown for 15 days on peptone broth. A. Seedlings grown in filtrates from race 1. B. Same as A except that filtrates were sterilized. C. Seedlings grown in filtrates from race 6. D. Seedlings grown in the noninoculated (control) solution.

and B). Ninety per cent of the seedlings grown in the filtrate from race 6 developed a root system similar to that of seedlings in the control solution (Fig. 5).

CONTROL OF DAMPING-OFF OF SOYBEANS

The intensification of soybean culture is likely to result in an increase of those isolates of *Rhizoctonia solani* that are virulent on this host, unless prevented by appropriate rotations and, perhaps, seed treatment. For this reason the effectiveness of various compounds applied to the seed in preventing damping-off were tested.

Plump, healthy, noncracked seed of Minsoy soybeans grown at University Farm in 1945 were treated with Arasan (tetramethyl thiuramdisulfide 50 per cent), Fermate (ferric dimethyldithiocarbamate), New Improved Ceresan (ethyl mercury phosphate 5 per cent), Cuprocide (cuprous oxide 96.5 per cent), Spergon (tetrachloro parabenzoquinone 98 per cent), Ceresan M (ethyl mercury p-toluene sulfonanilide 7.7 per cent), Leafox 200-A (zinc oxide 98.5 per cent), and Wettable Spergon (tetrachloro parabenzoquinone 48 per cent).

Cuprocide was applied at the rate of 3 oz. per bu., New Improved Ceresan at 1.5 oz. per bu., Spergon, Arasan, Fermate, and Ceresan M at 2 oz. per bu.; Wettable Spergon, 1 lb. to 25 gal. of water.

The field plan for both the spring (June 2, 1946) and the fall (August 28, 1946) experiments was a simple randomized block pattern. Each treatment was replicated four times. The rows were 6 ft. long, and the distance between rows and blocks was 3 ft. Fifty seeds were planted in each row. Two days prior to planting, inoculum of isolate 1 was put into the opened rows. Final notes on stand for both experiments were taken 14 days after planting.

All treatments, except Fermate, more than doubled seedling emergence

TABLE 4.—*Seedling emergence from seed of Minsoy soybeans treated with eight fungicides and planted in soil infested with isolate 1 of Rhizoctonia solani*

Treatment	Average number and percentage of plants that emerged			
	Spring		Fall	
	Number[a]	Per cent	Number[a]	Per cent
Arasan	31	62	27	55
Spergon	46	92	43	86
Fermate	31	62	20	40
New Improved Ceresan	41	82	42	84
Ceresan M	42	84	39	78
Cuprocide	32	64	29	57
Wettable Spergon	39	78	29	58
Leafox 200-A	34	68	30	60
Control	16	32	10	20
L. S. D.	4.6	.....	2.8	.....

[a] Perfect emergence would be 50.

(Table 4). However, preemergence damping-off caused by isolate 1 was best controlled by Spergon; Ceresan M and New Improved Ceresan also significantly increased seedling emergence. Analysis of variance shows that the variance between all treatments is highly significant at the 5 per cent level (Table 4).

DISCUSSION AND CONCLUSIONS

Rhizoctonia solani parasitizes soybeans and causes damping-off. Germinating seeds and seedlings of soybeans may be injured by *Rhizoctonia solani* even when the fungus hyphae do not parasitize the soybean tissues. Substances toxic to soybean occur in filtrates from the fungus hyphae and exert their effect at some distance from the host cells. If *Rhizoctonia solani* infests the soil in which soybeans are grown, the harmful effects of the filtrates may cause damping-off and reduce the stands. The significance of the filtrates, however, in causing damping-off and necrotic lesions on stems and roots of plants grown in the field was not investigated.

The pathogenicity of *Rhizoctonia solani* on soybeans is generalized rather than specific. This conclusion was based on results obtained from pathogenicity studies of only 16 races of Rhizoctonia on only five soybean varieties and may not remain valid after more races and varieties or selections are tested. A generalized rather than a specific type of pathogenicity was also reported by Tsiang (13) in his experiments with several races of Rhizoctonia on 12 varieties of flax grown in the field.

Chemical seed treatment, use of resistant varieties, and crop rotation are three possible methods for controlling plant disease. The investigations in this paper were confined to seed treatments for controlling Rhizoctonia. The results from the experiments indicate that damping-off of soybeans in field plots inoculated with Rhizoctonia was best controlled with Spergon (tetrachloro parabenzoquinone) and Ceresan M (ethyl mercury p-toluene sulfonanilide). Because a root-rot epidemic did not occur in Minnesota

during this investigation it was not possible to determine the efficacy of the seed treatments under natural conditions.

SUMMARY

Although no disease epidemics have occurred on soybeans in Minnesota, some pathogens, especially *Rhizoctonia solani*, are a potential threat to the crop.

Two factors that may affect the incidence of soybean root rots are the seasonal variation of environment and physiologic races of Rhizoctonia.

In controlled soil temperature experiments races 14 and 1 caused most damping-off at 25°–29° C.

Field and laboratory studies indicated that certain isolates can parasitize germinating seeds and also seedlings of soybeans by acting on the host tissue at a distance.

The diluted filtrates of the strongly pathogenic isolate 1 reduced seed germination and inhibited seedling root development of Minsoy soybeans.

Seed germination and seedling root development were not affected by the diluted filtrates of the weakly pathogenic isolate 6.

The five varieties of soybeans tested, Habaro, Bansei, Minsoy, Manchu Wisconsin No. 606, and Manchu Wisconsin No. 3, were equally susceptible to various isolates of Rhizoctonia except for minor differences.

Damping-off caused by *Rhizoctonia solani* was considerably reduced in experimental plots by treating the soybean seed with Spergon or Ceresan M.

UNIVERSITY FARM

ST. PAUL, MINNESOTA

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RED NODE, A VIRUS DISEASE OF BEANS

H. Rex Thomas and W. J. Zaumeyer

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In 1938 Pinto bean plants with reddish discolored nodes and pulvini and malformed pods were observed by the junior writer in several commercial bean fields in northeastern Colorado. The symptoms on the pods ranged from depressed reddish ringed patterns to large reddish sunken areas. In extreme cases complete collapse of the pod was noted. This condition also was observed in other varieties and hybrids in the experimental bean plantings at Greeley, Colorado. At the time the disease was first observed its cause was not known, and it was referred to as a physiological breakdown (5). The disease was worse along the edges of fields in proximity to ditches and fence rows. This together with other evidence indicated that possibly it was caused by a virus. Subsequent to 1938, similar diseased bean plants were observed in western Colorado where the disease was causing considerable damage in some Pinto bean fields. In 1942 the same disease was very common on snap and dry beans in Wyoming and southern Idaho. In some late plantings of snap beans in Wyoming, the last-formed pods on the plants were so severely damaged that a reduction in yield resulted; possibly a late migration of an insect vector may have caused the widespread transmission of the disease. Virgin (12) in 1943 described the same disease in Idaho, naming it red node, and reported that preliminary evidence indicated that it was caused by a virus closely related to yellow bean mosaic virus. Since that time it has occurred erratically, and only in a few instances has it been of economic importance.

In 1947 during an investigation of strains of yellow bean mosaic virus, in which red-node virus was included, it was found that the latter was infectious to cucumber and tobacco and hence was not related to the yellow bean mosaic virus group. In order to identify the red-node virus, it was compared with certain viruses reported to be infectious on tobacco and to cause a systemic necrosis of bean. The results of these studies indicated that the red-node virus was a strain of tobacco-streak virus, and hence it is being given the trinomial *Annulus orae* H. var. *phaseoli* var. nov.

MATERIALS AND METHODS

All the investigations reported here were made in the greenhouse at Beltsville, Maryland, at temperatures ranging from 21° to 27° C. The greenhouses were kept reasonably free of insects by frequent fumigation.

The culture of red-node virus used for the preliminary studies during the early part of 1948 was obtained from an infected plant of Landreth Stringless Green Pod variety collected at Twin Falls, Idaho, in August 1947. For the major part of the work, which was done from October 1948 to August 1949, a culture obtained in September 1948 from naturally infected

Pinto bean plants collected near Greeley, Colorado, was used. Several other cultures obtained from Colorado, Wyoming, and Idaho in 1948 were discarded because they appeared to be similar when inoculated to beans and tobacco in the greenhouse.

Cultures of viruses reported to be systemically infectious on tobacco and to cause a systemic necrosis on beans in the United States were obtained as follows: tobacco ring-spot, *Annulus tabaci* var. *virginiensis* H., and tobacco-streak, *Annulus orae* H., from H. H. McKinney, Plant Industry Station, Beltsville, Maryland; tomato ring-spot, *Annulus zonatus* H., from W. C. Price, University of Pittsburgh; alfalfa virus N from F. P. McWhorter, Oregon State College; and cultures of two strains of tobacco-streak virus designated B and D from R. W. Fulton, University of Wisconsin. Stock cultures of the viruses were maintained on Pinto U. I. No. 78[1] variety of bean and on the Samsun variety of Turkish tobacco. Unless otherwise indicated, "streak" refers to strain B obtained from Wisconsin.

All plants were inoculated in early stages of growth. The beans were inoculated when the primary leaves were not quite fully expanded, 10 to 12 days after planting. Transfers on beans were made approximately every 2 weeks. Carborundum powder No. 320 was used as an abrasive. In general the methods used were similar to those employed in previous investigations (15). In certain of the host-range studies the infected bean leaves were ground with 0.01 M sodium sulphite which, along with other reducing agents, had been shown by Fulton (4) to reduce the rapid inactivation of the tobacco-streak virus. Because it was found in limited tests on beans that satisfactory infection was obtained by using fresh inoculum, the use of the reducing agent was soon discontinued.

SYMPTOMS

Beans. The first noticeable symptoms of red node in the field are a reddish discoloration of the nodes (Fig. 1, A) of the stem and pulvini of the leaves and leaflets, together with reddish concentric ringed patterns on the pods. These may vary from small indistinct markings to large reddish sunken blotches which may involve most of the pod (Fig. 1, B, C). In extreme cases there is a decided shriveling of the pod (Fig. 1, D), without seed formation. The reddish discoloration of the nodes may be limited to a few on each plant. Discoloration of the stem, growing point, veins, and veinlets of the leaves may occur. Usually infected plants do not die but are stunted and frequently break off at one of the weakened infected nodes. Occasionally, however, early infected plants develop systemic necrosis and die. An apparent recovery stage, manifested by the development of a mild mottle on the newly formed leaves, has been observed. This growth is not normal but consists of numerous dwarfed, mildly mottled leaves. Such plants are decidedly stunted.

[1] The initials "U. I." indicate that the variety was originated by the University of Idaho.

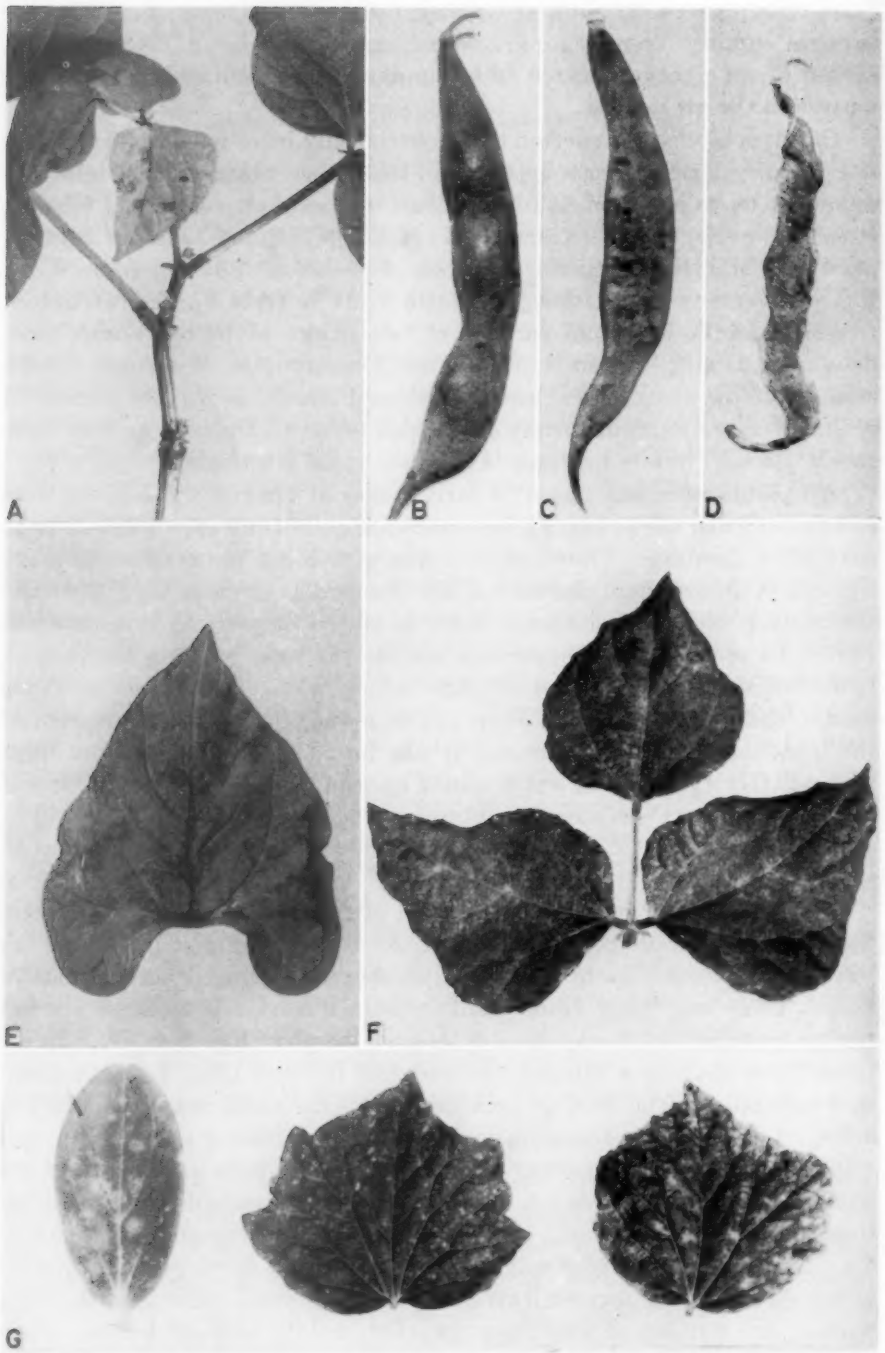

FIG. 1. Symptoms of red-node virus produced on plants inoculated in the greenhouse. Pinto U. I. No. 78: A. Reddened nodes and pulvini and systemic necrotic ringlike lesions on young trifoliate leaf. B, C. Infected pods with ringlike patterns. D. Shriveled pod. E. Underside of inoculated leaf showing primary lesions. F. Mottle on trifoliate leaf from plant with "recovery" symptoms. G. Primary lesions on cotyledon and yellow stipple and mottle on leaves of Cubit cucumber.

Usually some of the pods on a plant are not infected. When the affected area on a pod is large and sunken the pods become malformed. Often the more advanced pods develop seeds even when they become infected, but younger pods on a cluster may dry and shrivel. The infected seeds are often discolored, with concentric ringlike markings.

In the greenhouse large, irregular, necrotic lesions develop in 2 to 3 days on inoculated bean leaves (Fig. 1, E). When viewed from the under side the veins appear reddened before the interveinal tissue has collapsed. The discoloration progresses into the stem and growing point and in severe cases the young plant may die within 5 days. Plants that are not killed have reddened nodes and pulvini and reddish necrotic streaks on the stem and are severely stunted. Frequently the new leaves develop small, reddish-brown, concentric ringed patterns (Fig. 1, A) and are malformed. The older leaves are dwarfed and thickened and have reddish veins. Occasionally a mild mottle develops on the young leaves (Fig. 1, F). As in the field, many of the pods borne on the diseased plants are normal, but a few develop reddish markings and sometimes the younger pods shrivel and drop off.

Other hosts. The symptoms on certain hosts artificially inoculated with the red-node virus in the greenhouse are described.

On most of the leguminous hosts the red-node virus caused a necrosis. On guar, *Cyamopsis tetragonaloba* (L.) Taub., local lesions were produced on the inoculated leaves, but no systemic symptoms were evident. On white sweet clover (*Melilotus alba* Desr.) and crimson clover, *Trifolium incarnatum* L., only mottling and stunting of the plant occurred.

Large chlorotic primary lesions were occasionally observed on the inoculated cotyledons of cucumber, but usually a yellow stipple mottle on the young leaves was the first sign of infection (Fig. 1, G). This was followed by enlargement of the yellowed areas until a general mottle occurred (Fig. 1, G).

Inoculated Turkish tobacco leaves usually had only one or two local lesions per leaf. These developed within 3 to 5 days after inoculation and varied from a solid necrotic lesion to concentric ring patterns approximately 0.5 to 2 cm. in diameter (Fig. 2, A). The systemic symptoms appeared within 5 to 7 days after inoculation and consisted of whitish to brown, necrotic, ringed or "oak-leaf" patterns (Fig. 2, C). The new leaves that developed after the appearance of these symptoms had no signs of infection except that they were stunted (Fig. 3, B). Occasionally systemic necrosis developed on older plants.

Primary ringlike necrotic lesions were noted on the inoculated leaves of jimsonweed (Fig. 2, B), but more frequently a mild mottle was the only evidence of infection. At other times systemic whitish necrotic markings and patterns occurred. The first few leaves formed after inoculation were mildly mottled, but the subsequent growth was symptomless.

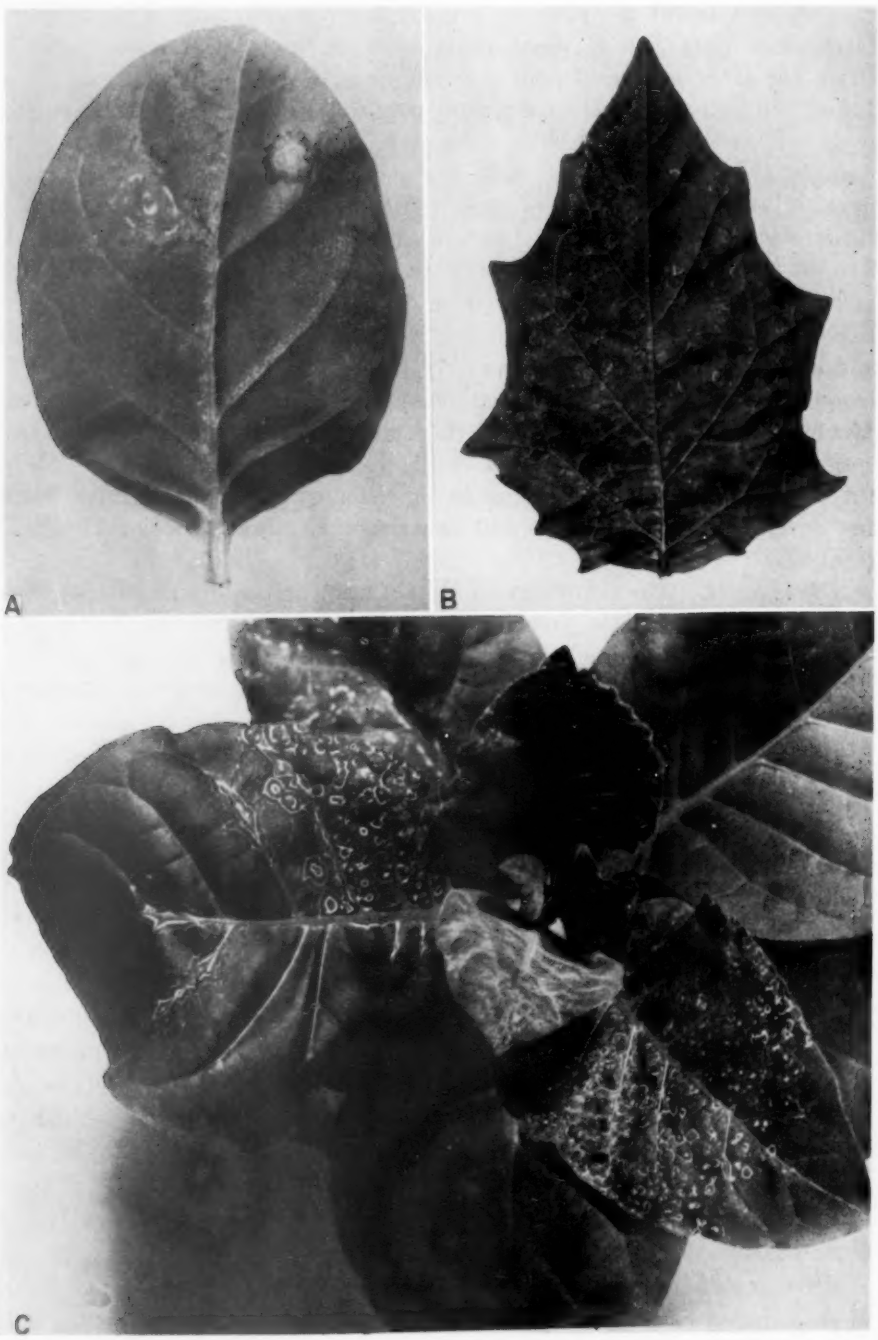

FIG. 2. Primary lesions of red-node virus on inoculated leaf of: A, Turkish tobacco, and B, jimsonweed. C. Systemic ringlike markings on red-node-infected Turkish tobacco plant.

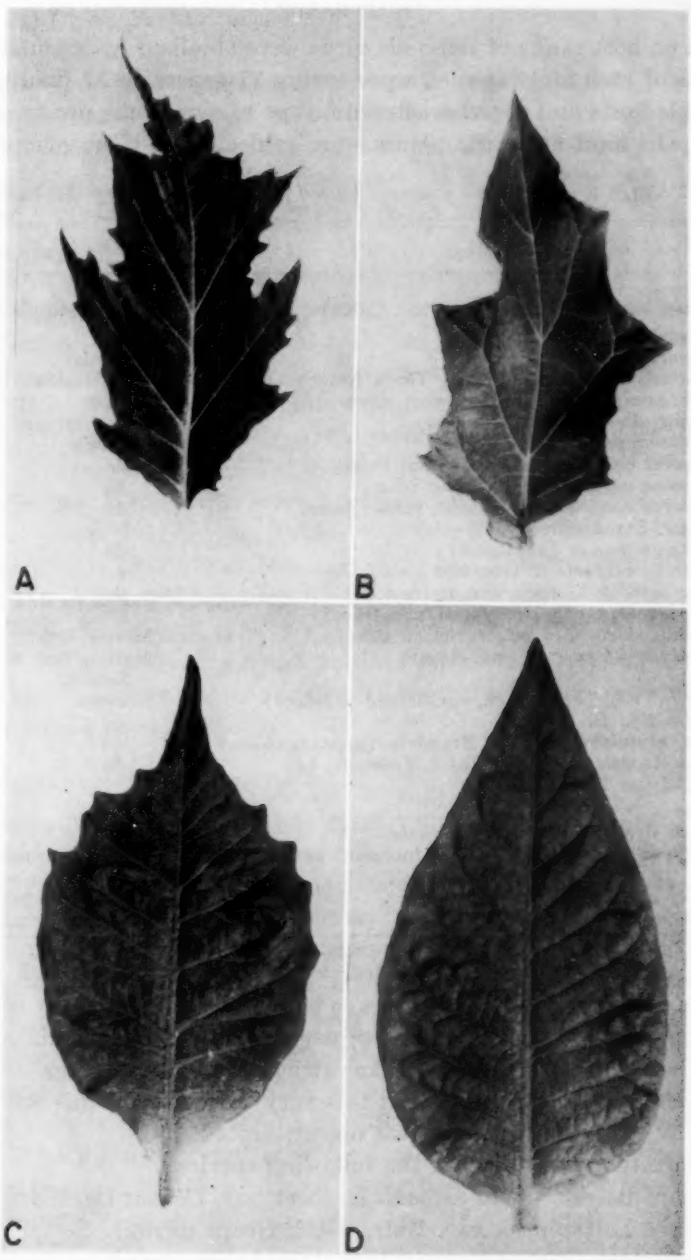

FIG. 3. Symptoms on tolerant leaves of red-node- and tobacco-streak-infected plants: A. Holly-leaf margin on tobacco-streak-infected jimsonweed. B. Leaf from red-node-infected jimsonweed plant. C. Holly-leaf margin on tolerant leaf from tobacco-streak-infected Turkish tobacco plant. D. Tolerant leaf from red-node-infected Turkish tobacco plant.

HOST RANGE

Data on host range of red-node virus were obtained by inoculating 5 to 10 plants of each of 41 species representing 27 genera in 11 families. The susceptible hosts and the characteristic type of symptoms produced are in table 1. In most cases the plants were reinoculated if no symptoms appeared. Because of the rapid inactivation of the virus in fresh juice extractions, the failure of some species to become infected does not necessarily prove that these plants are immune; perhaps they are more difficult to infect. The recent increase in the knowledge of the host range of tobacco-streak virus by Fulton (3) makes this very evident. In this study transmission by grafting or insects was not attempted.

TABLE 1.—*Symptoms produced by red-node virus on susceptible hosts*

Host	Symptoms
Cucurbitaceae	
Cucumis sativus L. (cucumber var. Cubit)	Yellow stipple mottle
Leguminosae	
Crotalaria spectabilis Roth	Necrosis
Cyamopsis tetragonoloba (L.) Taub. (guar)	Local lesions
Melilotus alba Desr. (white sweet clover)	Mottle
Lupinus albus L. (white lupine)	Local lesions
Lupinus angustifolius L.	Necrosis
Phaseolus aconitifolius Jacq. (mat bean)	do
Phaseolus aureus Roxb. (mung bean)	do
Phaseolus coccineus L. (scarlet runner bean, var. Streamliner)	do
Phaseolus mungo (urd bean)	do
Phaseolus vulgaris L. (common bean)	do
Pisum sativum L. (pea, var. Perfection)	do
Soja max (L.) Piper (soybean, var. Biloxi)	do
Trifolium incarnatum L. (crimson clover)	Mottle
Trifolium pratense L. (red clover)	Mottle, fine necrotic flecking
Vicia faba L. (broadbean, var. Broad Windsor)	Necrosis
Vicia sativa L. (vetch)	do
Vigna sesquipedalis (L.) Fruwirth (asparagus-bean)	do
Vigna sinensis (Torner) Hassk. (cowpea, var. Chinese Red)	do
Solanaceae	
Datura stramonium L. (jimsonweed)	Mottle
Nicotiana tabacum L. (Turkish tobacco, var. Samsun)	Necrotic primary lesions, systemic necrotic rings plus recovery
Nicotiana rustica L.	Necrotic primary lesions

No infection was noted on the following species:

Chenopodiaceae. *Beta vulgaris* L. (beet, var. Detroit Dark Red), *Spinacia oleracea* L. (spinach var. Extra Dark Green Savoy).

Compositae. *Zinnia elegans* Jacq. (zinnia var. Sunshine Tint).

Cruciferae. *Brassica oleracea* var. *botrytis* L. (broccoli, var. Italian Green Sprouting).

Leguminosae. *Canavalia ensiformis* (L.) DC. (jack bean), *Medicago sativa* L. (alfalfa var. Kansas common), *Phaseolus angularis* (Willd.) W. F. Wight (adzuke bean), *Phaseolus calcaratus* Roxb. (rice bean), *Phaseolus*

lathyroides L., *Sesbania exaltata* (Raf.) Cory, *Trifolium repens* L. (white clover).

Phytolaccaceae. *Phytolacca americana* L. (pokeweed).

Portulacaceae. *Portulaca oleracea* L. (purslane).

Ranunculaceae. *Aquilegia canadensis* var. Hybrida Hort.

Solanaceae. *Lycopersicon esculentum* Mill. (tomato var. Rutgers), *Nicotiana glutinosa* L., *Petunia hybrida* Vilm. (petunia, var. Martha Washington).

Tropaeolaceae. *Tropaeolum majus* L. (nasturtium, var. Golden Globe).

Umbelliferae. *Apium graveolens* L. (celery, var. Golden Plume).

The following 25 varieties or strains of snap and dry beans were inoculated with the red-node and streak viruses: Brittle Wax, Cherokee Wax, Florida Belle, Full Measure, Giant Stringless Green Pod, Great Northern U. I. No. 31, Idaho Refugee, Keeney's Stringless Green Refugee, Kentucky Wonder, Kentucky Wonder Wax No. 765,[2] Kentucky Wonder White, Kentucky Wonder White No. 780,[2] Kentucky Wonder No. 814,[2] Landreth Stringless Green Pod, Logan, Pencil Pod Black Wax, Pinto U. I. No. 78, Puregold, Red Kidney, Red Mexican U. I. No. 3, Rival, Sensation Green Pod, Sensation Refugee No. 1066, Sure Crop, and Unrivalled Wax. A small number of plants of each variety was tested and results were recorded after 11 days. All varieties developed primary lesions and most of them developed reddish nodes and stem necrosis. In general all the varieties reacted similarly to the two viruses.

COMPARISON OF THE PROPERTIES OF RED-NODE AND STREAK VIRUSES

Expressed juice from young infected Pinto U. I. No. 78 plants was used for all the studies of physical properties. The results were based on primary lesions produced on this same variety of beans. Guar was used in a few tests, but it was not so satisfactory as Pinto bean.

Considerable difficulty was experienced in obtaining satisfactory thermal inactivation end points for red-node and streak viruses because of their rapid inactivation *in vitro*. To help compensate for this, separate virus extractions were made for each temperature. Less than 20 minutes usually elapsed from the time of extraction to inoculation. Ten plants were used as a test unit, and each test was repeated three times. Inoculations were also made with the unheated juice from each extraction to serve as a check on the infectivity of the virus. The thermal inactivation point for red-node virus was between 56° and 58° C. and that for streak virus between 54° and 56° C. when heated for 10 min. (Table 2).

Both red-node and streak viruses withstood dilution of 1 to 500 but not 1 to 1000 (Table 2).

Red-node virus was infectious after storage *in vitro* at 18° C. for more than 24 hr. but less than 48 hours, while streak was infectious for less than

[2] Stocks maintained at Beltsville, Maryland, for differentiating races of bean rust.

TABLE 2.—*Comparison of thermal inactivation points and tolerance to dilution of red-node and streak viruses as determined by production of primary lesions on the Pinto U. I. No. 78 variety of bean*

Thermal inactivation[a]			Tolerance to dilution[b]		
Temperature (°C)	Red node	Streak	Dilution	Red node	Streak
	Total lesions	Total lesions		Total lesions	Total lesions
	Number	*Number*		*Number*	*Number*
Unheated	Numerous	Numerous	Undiluted	Numerous	Numerous
50	Numerous	Numerous	1 – 100	6	15
52	185	115	1 – 200	3	5
54	43	28	1 – 500	1	2
56	7	0	1 – 1000	0	0
58	0	.....			
60	0	.....			

[a] Determined by lesions produced on 60 leaves.
[b] Determined by lesions produced on 20 leaves.

TABLE 3.—*Comparison of tolerance to longevity in vitro and to drying of red-node and streak virus as determined by production of primary lesions on 20 leaves of Pinto U.I. No. 78 variety of bean*

Longevity *in vitro*			Longevity in dry tissue		
Storage period	Red-node lesions per leaf	Streak lesions per leaf	Storage period	Red-node virus recovered	Streak virus recovered
Minutes	*Number*	*Number*	*Days*		
0	37.0	26	1	+[c]	+
10[a]	28.0	15	2	+	+
20	8.0	6	3	+	+
30	1.0	4	7	+	+
40	1.0	2	14	+	+
50	0.5	1	30	+	+
60	0.1	1	90	0	+
24 hr.[b]	0.05	0	.....	.....	.....
48 hr.[b]	0.0	0	120	0	0

[a] Room temperature approximately 22° C.
[b] Stored at 18° C. in darkness.
[c] Based on presence or absence of local lesions on 10 primary leaves.

24 hr. (Table 3). Inactivation of both viruses was rapid within 20 min. after extraction (Table 3).

Infected leaves were dried at room temperature, 20° to 25° C., and became crisp after 48 hr. After various periods of storage the dried material was ground with water in a mortar and inoculated to bean leaves. Infection with both red-node and streak viruses occurred after storage for 30 days. The streak virus was infectious after storage for 90 days (Table 3).

RELATIONSHIP OF TEMPERATURE TO FORMATION OF PRIMARY LESIONS OF RED-NODE AND STREAK VIRUSES

The influence of temperature on the development of primary lesions of red-node and streak viruses was determined by placing two 4-in. pots of

five bean plants each in each of five controlled temperature chambers (Table 4). At 26° C. only 2 days' time was required for the development of lesions for the two viruses as compared with 5 days at 14° and 18° and 3 days at 22°. At 10° no lesions appeared within 8 days.

SEED TRANSMISSION

Seeds from pods showing symptoms of red-node infection were gathered in a severely infected field of Pinto U. I. No. 111 beans near Greeley, Colorado. No infection was observed on 146 greenhouse-grown plants from these seeds. No infection was found on 239 greenhouse-grown plants from seed collected from red-node-infected pods of Great Northern U. I. No. 123 plants grown near Bridger, Montana.

CROSS-PROTECTION STUDIES

Young mottled leaves of three jimsonweed plants infected with red-node virus, as well as young leaves of three healthy plants, were inoculated with the tobacco ring-spot virus. Typical tobacco ring-spot lesions developed on the inoculated leaves of both groups of plants. These results indicate that red-node and tobacco ring spot are unrelated.

Young leaves of two tobacco plants in the recovery stage of red-node infection and similar leaves of two healthy plants of the same age were inoculated with streak D virus. Corresponding leaves of the same plants were also inoculated with the red-node virus. No primary lesions of streak D or red-node viruses appeared on the leaves of the plants infected with red node. Both viruses produced primary lesions on the healthy plants.

Young leaves on eight jimsonweed plants infected with red-node virus and similar leaves on four healthy plants were inoculated with streak D virus. Since production of primary lesions on jimsonweed is inconsistent, final results were based on the appearance of systemic symptoms. Only one of the eight red-node-infected plants developed systemic symptoms typical of streak infection, but the four healthy jimsonweed plants became infected with streak.

Considerable difficulty was experienced in obtaining satisfactory cross-protection tests, because of the inconsistent results obtained when leaves of older plants were inoculated with the red-node and streak viruses. The experiments just described are suggestive that the red-node virus cross-protected against infection by the streak virus, indicating a relationship between the viruses. No conclusive data were obtained from repeated tests to determine whether the streak virus protected against infection of the red-node virus.

INFLUENCE OF SOURCE OF INOCULUM ON INFECTIVITY OF THE RED-NODE VIRUS

Early in the current investigations of the red-node virus, it was noticed that beans were readily infected with red node when inoculated with expressed juice from infected beans, but that tobacco was difficult to infect with this source of inoculum regardless of the age of the plants. If, how-

ever, the inoculum was obtained from infected tobacco, infection in tobacco occurred more readily but beans were difficult to infect. Infection of jimsonweed with inoculum obtained from infected beans usually produced no primary lesions but only mild mottle. When jimsonweed was inoculated with expressed juice from red-node-infected plants of this host, numerous primary lesions as well as severe systemic mottled symptoms developed.

DISTRIBUTION OF RED-NODE-INFECTED PLANTS IN THE FIELD

Observations on the distribution of red-node-infected plants in the field have been limited. In such a study in three large fields near Greeley, Colorado, in 1948 it was found that as high as 100 per cent of the plants were infected at the edge of the field but that the percentage of diseased plants decreased rapidly toward the interior of the field, often becoming as low as 3 to 5 per cent in 200 ft. of row. In a few fields, however, the infection appeared more or less uniform throughout. The distribution of infected plants in the field suggested insect transmission from infected weeds growing along fencerows and ditch banks.

RELATION OF RED-NODE VIRUS TO CERTAIN OTHER VIRUSES

Comparative inoculations with tobacco-streak and red-node viruses suggested a close relationship between these two viruses on the basis of symptomatology. Red-node and tobacco-streak viruses produced identical symptoms in most of the bean varieties tested. The symptoms produced on numerous other hosts were very similar and in many cases identical.

Certain consistent differences, however, were noted between red-node and the three strains of streak viruses that were studied. The virus of red node was never transmitted to *Nicotiana glutinosa*, whereas the streak virus strains were transmitted fairly consistently to this host. Although red-node and streak viruses produced similar primary lesions and systemic symptoms on Turkish tobacco, infection from diseased tobacco to healthy tobacco seedlings was much more readily obtained with the streak virus than with the red-node virus. The streak virus was usually reisolated from the tolerant leaves of streak-infected tobacco, but numerous attempts to reisolate the red-node virus from tolerant leaves of red-node-infected plants were negative. On tobacco, systemic infection with streak virus follows the development of primary lesions rather consistently, but this is not the case with red-node virus. The tolerant leaves of streak-infected Turkish tobacco plants develop a ragged margin or "holly-leaf" effect (Fig. 3, C). This was typical of all three strains of streak virus and was observed from December to June. This symptom was not observed on red-node-infected Turkish tobacco plants (Fig. 3, D). At first both viruses produce a mild mottle on jimsonweed. The new growth of streak-infected jimsonweed plants developed ragged margins or holly-leaf effect and roughened and thickened leaves (Fig. 3, A), in contrast with the leaves of red-node-infected

plants, which were stunted but had a normal surface and margin (Fig. 3, B).

Each of nine varieties of soybean were inoculated separately with the red-node and streak viruses, and on six of these similar symptoms were produced. Lincoln and Burdette varieties developed systemic necrosis symptoms and died. Arksoy, Ralsoy, Illini, and Gibson developed less intense systemic necrosis but were not killed. The other three varieties, CNS, Biloxi, and Cherokee, responded differently.

A second planting of these last three varieties together with the Arksoy variety was made to determine whether similar responses would develop. Each variety was again inoculated separately with red-node, streak B, and streak D viruses. Plants of CNS variety inoculated with red-node virus, although stunted and mottled, produced no systemic necrosis, whereas plants infected with the two streak virus strains developed terminal necrosis and died (Table 4). Plants of Biloxi and Cherokee varieties inoculated

TABLE 4.—*Symptoms produced on several varieties of soybeans by red node, streak "B," and "D" viruses*

Variety	Symptoms produced by		
	Red node	Streak B	Streak D
Arksoy	Necrosis on inoculated leaves	Stem necrosis, stunt and mottle	Stem necrosis, mottle
CNS	Stunt, mottle	Death	Death
Biloxi	Death	Necrosis on inoculated leaves, mild mottle	Necrosis on inoculated leaves
Cherokee	Death	Necrosis on inoculated leaves	Necrosis on inoculated leaves

with red-node virus also developed systemic necrosis and died. Plants of these same varieties infected with streak B and D virus strains showed only veinal necrosis on the inoculated leaves and were not stunted. Plants of Arksoy variety developed stem necrosis when infected with the streak or the red-node virus.

Inoculations were made to several hosts with the viruses of tobacco ring spot, tomato ring spot, alfalfa N, and red node. The primary lesions produced on beans by the tobacco and tomato ring-spot viruses were circular and smaller than the large, irregular, spreading lesions produced by the red-node virus. Tobacco ring-spot virus caused solid primary lesions on jimsonweed, in contrast to the circular or ringlike necrotic lesions produced on plants inoculated with the red-node virus. Red-node virus was also distinguished from the tomato ring-spot virus by being noninfectious to tomatoes under conditions of the present investigations, while the latter was readily infectious to this host. Red-node virus was not infectious on *Nicotiana glutinosa* as were the other viruses. On Turkish tobacco the alfalfa N virus produced yellow chlorotic primary lesions, in contrast to the concentric ring patterns produced by the red-node virus.

Potato yellow dwarf virus, *Aureogenus vastans* (H.) Black, is characterized by being difficult to transmit mechanically to most of its hosts (2). Red-node-infected bean seedlings were sent to L. M. Black of the Brooklyn Botanic Garden for comparison of this virus with the potato yellow dwarf virus. He reported on the basis of symptoms produced on *Nicotiana rustica*, that these viruses are not related.

DISCUSSION

It has been shown previously that the viruses of tobacco ring spot (14), tomato ring spot (7), tobacco streak (1), stipple streak (13), Bergerac ring spot, *Annulus bergerac* H. (9), and alfalfa virus N (6) cause systemic necrosis of beans.

Red-node virus can be distinguished from stipple streak, Bergerac ring-spot, and tobacco ring-spot viruses by differences in their physical properties. The red-node virus has a thermal inactivation point between 56° and 58° C. and a dilution end point between 1–500 and 1–1000. Stipple streak, Bergerac ring-spot, and tobacco ring-spot viruses are inactivated by heating for 10 min. between 85° and 90°, at 80°, and at 68°, respectively, and have dilution end points of 1–100,000, 1–100, and 1–10,000, respectively.

The thermal inactivation points of tobacco-streak, tomato ring-spot, potato yellow dwarf, and alfalfa N viruses are in somewhat close agreement with those of the red-node virus. These are 52° to 54° C. for the tobacco-streak virus, 55° to 60° for the tomato ring-spot virus, and 50° to 52° for the potato yellow dwarf virus (2), and as mentioned earlier, 56° to 58° C. for red-node virus. The thermal inactivation point of alfalfa N is reported to be 10 to 15 degrees lower than those previously reported for strains of the alfalfa virus (6). As described elsewhere, tomato ring-spot and potato yellow dwarf viruses can be differentiated from red-node virus on the basis of symptomatology on different hosts.

No control measures can be recommended at present, because the natural source of the virus and the means of transmission are not yet known. Skiles and Thomas (8) reported that preliminary greenhouse studies indicated that the red-node virus is seed-transmitted. Evidence obtained in the present investigations indicates that the virus is not seed-transmitted.

The frequent concentration of infection along the edges of bean fields strongly suggests insect transmission from susceptible cultivated or wild host plants. Certain crop and ornamental plants as well as weeds have been shown by Thomas (11) to be susceptible to infection by the red-node virus. Although the host range of tobacco streak is very extensive as shown by Fulton (3), so far as is known, no vector for this virus has been found.

The development of bean varieties resistant to red node may be feasible. No resistant varieties were found among a limited number tested. However, Skiles and Thomas (8) and Thomas (10) reported that in greenhouse tests Kentucky Wonder White No. 780 and Kentucky Wonder Brown No. 814 showed no evidence of infection when mechanically inoculated with the

red-node virus. This is not in agreement with the results presented here.

Similarity of symptoms produced on beans, tobacco, and jimsonweed, of properties, and of limited cross-protection studies suggest that the red-node virus is related to tobacco streak. Certain differences in the behavior of the red-node virus, such as not infecting *Nicotiana glutinosa*, and not inducing a holly-leaf pattern on the tolerant leaves of infected Turkish tobacco and jimsonweed, probably warrant a variety or strain distinction. The name *Annulus orae* H. var. *phaseoli* is proposed.

TECHNICAL DESCRIPTION OF THE VIRUS

Annulus orae var. **phaseoli** var. nov.

Common name: Red-node virus.

Variety name: Referring to occurrence on *Phaseolus vulgaris* L.

Host reactions: Primary necrotic lesions induced on Pinto U. I. No. 78 and other varieties of beans in 2 to 3 days, subsequent development of systemic stem necrosis and reddened nodes. In tobacco local and systemic necrosis in 3 to 7 days, with necrotic spot, line, and ringlike lesions. Tolerant leaves stunted, but not typically mottled. Reinoculation does not induce necrotic primary lesions. Infectious to jimsonweed, cucumber, cowpeas, peas, white sweet clover, red clover, and crimson clover. Not infectious to *Nicotiana glutinosa* or tomato.

Transmission: Readily transmitted by inoculation with expressed juice rubbed on leaves. No insect vectors known. Appears not to be seed-transmitted.

Physical properties: Inactivated between 56° and 58° C. in 10 min. in plant juices. Dilution end point in fresh plant extract from infected bean between 500 and 1,000 ×. Resisted aging for 24 hr. at 18° C., but inactive after 48 hr. in plant juices. Rapid inactivation of virus within 20 min. in plant juices. Resisted drying at room temperatures 20° to 25° for 30 days but not 90 days.

Immunological relations: Limited evidence that tolerant leaves on red-node-infected tobacco and jimsonweed are protected against infection by tobacco-streak virus.

Differences from type species: No infection of *Nicotiana glutinosa*. Lack of holly-leaf pattern of tolerant leaves of red-node-infected Turkish tobacco and jimsonweed. Two degrees higher thermal inactivation end point.

Type locality: Colorado, Idaho, and Wyoming.

SUMMARY

Red node, a previously reported virus disease of bean, occurs erratically in the bean-growing areas of the Mountain States. It causes reddish discolored nodes of the stem and pulvini of the leaves and leaflets together with reddish concentric ringed patterns on the pods.

The red-node virus was found to be infectious by mechanical transmis-

sion with carborundum to tobacco, cucumber, jimsonweed, guar, pea, soybean, crimson clover, white sweet clover, red clover, broadbean, vetch, and cowpea as well as other hosts. No infection was obtained on tomato, *Nicotiana glutinosa*, white clover, and alfalfa.

Certain properties of the red-node and tobacco-streak viruses were compared. Red-node virus was inactivated between 56° and 58° C. and tobacco-streak virus between 54° and 56°. Both viruses tolerated a dilution of 1 to 500 but not 1 to 1,000. Red node was slightly infectious after storage for 24 hr. *in vitro* at 18° C., whereas tobacco streak was not. Both viruses rapidly lost their infectivity after storage for 20 min. *in vitro*. Red node resisted drying in leaf tissue for 30 days. Tobacco streak was infectious after 90 days' storage but not after 120 days.

There was no evidence of seed transmission.

No resistance to the red-node virus was found among 25 bean varieties or strains artificially inoculated.

Based on the similarity of properties, symptoms on beans, jimsonweed, Turkish tobacco, and on other hosts, and on limited cross-protection tests, it is concluded that the red-node virus is related to the tobacco-streak virus. Certain differences such as a slightly higher thermal inactivation end point, no infection on *Nicotiana glutinosa*, lack of a ragged leaf margin or holly-leaf pattern on the tolerant young growth of tobacco and jimsonweed in the "recovery" stage perhaps warrant a variety distinction. The trinomial *Annulus orae* H. var. *phaseoli* var. nov. is proposed.

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YELLOW STIPPLE, A VIRUS DISEASE OF BEAN

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In a shipment of mosaic-infected Stringless Black Valentine bean plants sent to the writers from southern Illinois in the fall of 1948, severely mottled and malformed pods (Fig. 1) were observed. The infected pods looked

Fig. 1. Serious mottle and malformation caused by virus complex on pods of Black Valentine bean received from Illinois.

like those affected with southern bean mosaic virus (*Marmor laesiofaciens* Zaumeyer and Harter) (13) or with pod mottle virus (*M. valvolarum* Zaumeyer and Thomas) (14). They were so severely affected that they were of no commercial value. In general, the plants were not stunted and their leaves were only mildly mottled, but some were considerably puckered and malformed.

Inoculations of beans of Pinto and other varieties with juice expressed from diseased pods produced, in some cases, local lesions resembling those typical of the disease caused by southern bean mosaic virus and pod mottle virus. The trifoliate leaves of the Pinto variety, which is resistant to sys-

temic infection by either of these two viruses, had a mild mottle at first and later a yellow stippling unlike the symptoms produced by any other virus heretofore reported on beans. The snap bean varieties inoculated with the juice from the trifoliate leaves of such affected Pinto plants had no yellow stippling and had only mild mottling which was difficult to differentiate definitely from that produced by certain previously described bean viruses. Purification studies proved that in the complex, both the virus of southern bean mosaic and that of pod mottle, as well as a new virus, were involved.

This paper reports the results of a study of this new virus in comparison with other bean viruses. Because of the distinctive yellow symptoms on leaves of certain dry bean varieties, the disease produced by this virus is called yellow stipple. In conformity with Holmes' system of virus classification (4), the name *Marmor flavopunctum* sp. nov. is proposed.

MATERIALS AND METHODS

The Stringless Black Valentine plants from which the yellow stipple virus was first isolated were supplied by the United States Seed Company, St. Louis, Missouri, from commercial bean fields in the vicinity of Anna and Villa Ridge, Illinois. Stock cultures of this virus were maintained on plants of Pinto U. I. No. 78[1] grown from virus-free seed produced in Colorado. All the studies were conducted at Beltsville, Maryland, in greenhouses maintained at temperatures usually ranging from 21° to 27° C. The plants were inoculated in the early stages of growth, about 10 to 14 days after planting. The primary leaves were dusted lightly with carborundum powder No. 320 and rubbed with the expressed virus juice, and the residue was removed by washing with water. When symptoms on the inoculated plants were very mild or absent, extracted juice from such plants was used to inoculate Pinto plants to determine the presence or absence of the virus.

The properties of the virus were determined by the methods previously used (12).

SYMPTOMS ON SNAP BEAN VARIETIES

The systemic mottle symptoms produced by yellow stipple virus are usually considerably milder than those produced by common bean mosaic virus, by its variant strain[2] (2, 8, 9), or by yellow bean mosaic virus. On some varieties and under some conditions it would be difficult to differentiate the symptoms produced by any of the others or from those produced by southern bean mosiac virus or by pod mottle virus. Leaves of snap bean varieties infected with yellow stipple virus are slightly malformed and mildly mottled, but do not have the severe puckering and blistering that frequently result from infection with the common bean mosaic virus or its variant strain[2] (2, 8, 9) (Fig. 2, B). Neither do they have the extreme yellow leaf blotching, general chlorosis, and malformation typical of yellow bean

[1] The initials "U. I." indicate that the strain was originated by the University of Idaho.

[2] Previously referred to as New York 15 mosaic and Burkholder's strain of common bean mosaic.

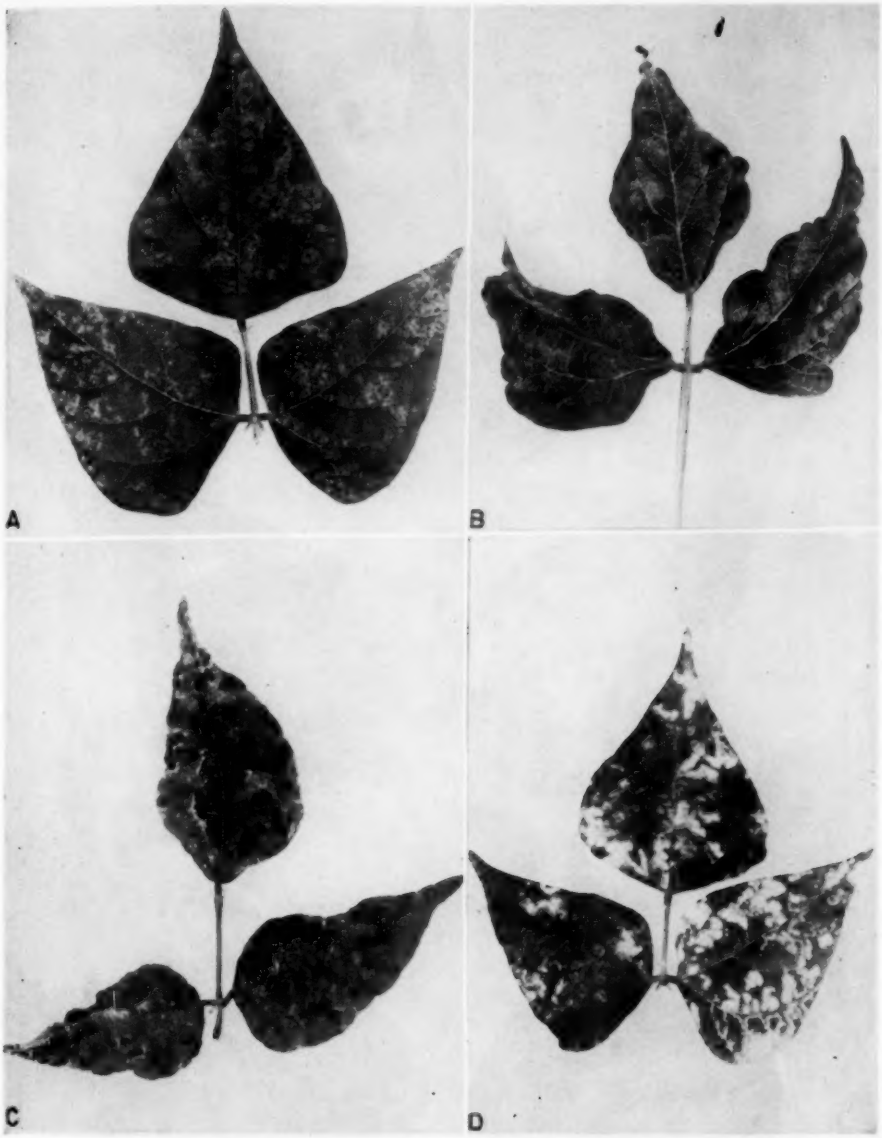

FIG. 2. Symptoms produced by several bean viruses on different bean varieties. A and D. Yellow stipple virus on Pinto U. I. No. 78. B. Common bean mosaic virus on Pinto. C. The gladiolus strain of yellow bean mosaic virus on Idaho Refugee.

mosaic (Fig. 2, C) in the greenhouse. The affected plants are not stunted, and their pods are not mottled and malformed like those of plants affected by pod mottle or southern bean mosaic virus.

SYMPTOMS ON DRY BEAN VARIETIES

On varieties such as Pinto and Great Northern, the yellow leaf stippling is very distinctive. Young trifoliate leaves of infected seedlings have at

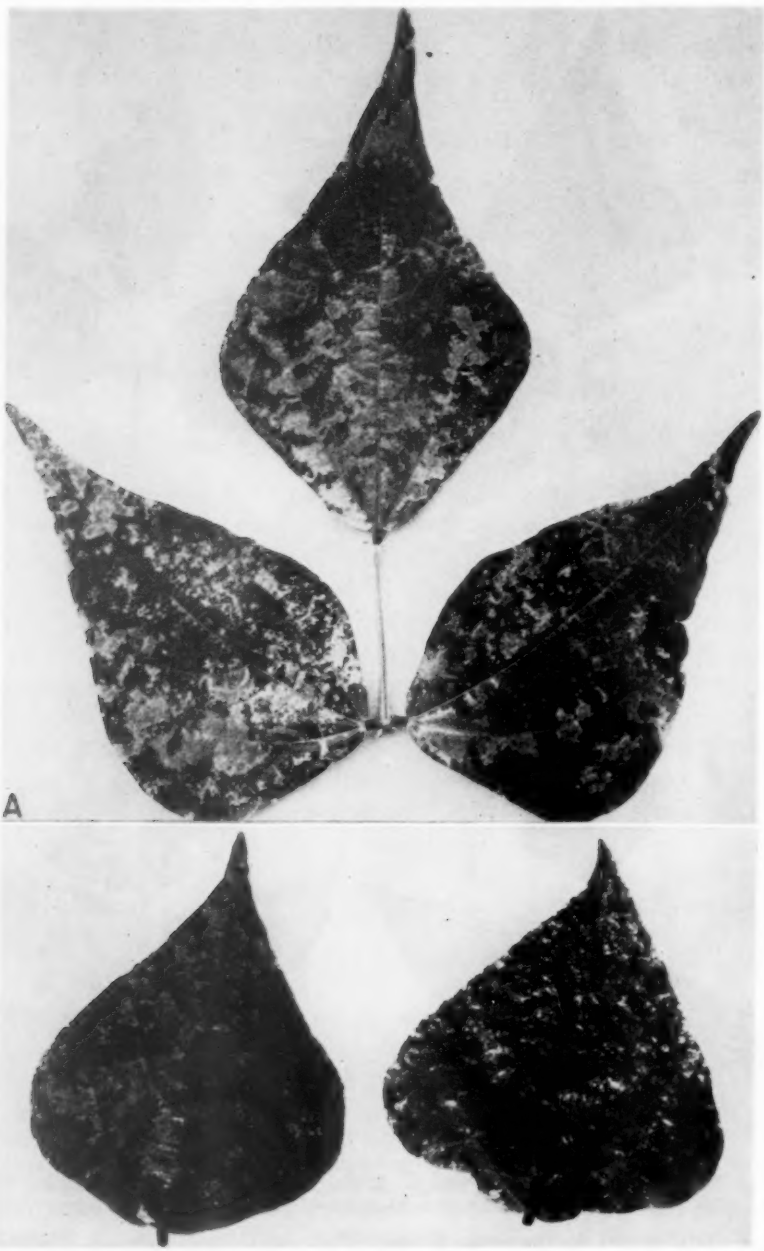

FIG. 3. A. "Yellow spot" on hybrid bean, produced by a heritable chlorophyll deficiency. B. Symptoms of a disease observed in Idaho in 1931, on leaflets of Great Northern U. I. No. 1. Note similarity of symptoms to those of yellow stipple disease.

first a mild mottle, and later small yellow chlorotic spots (Fig. 2, A) which may coalesce and form irregular patches as large as 10 mm. in diameter (Fig. 2, D). In some instances these areas may involve three-quarters of a

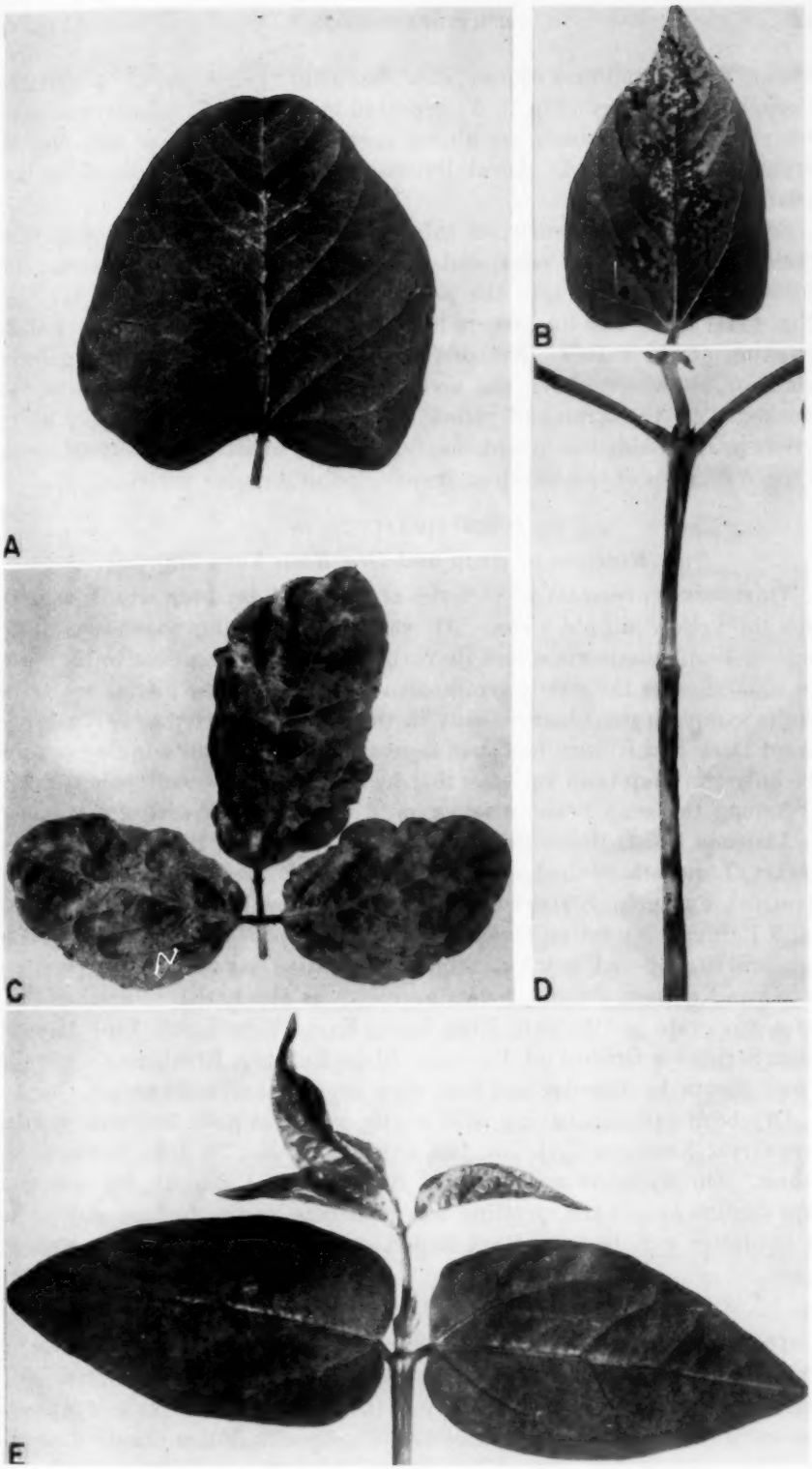

FIG. 4. Several species of Leguminosae infected with yellow stipple virus. A. Local lesions on Biloxi variety of soybean. B. Local lesions on tepary bean. C. Systemic mottle on Ralsoy variety of soybean. D. Stem necrosis on Pinto U. I. No. 78. E. Local lesions and systemic necrosis on Catjang type of cowpea.

leaflet. These symptoms appear identical with "yellow spot," a heritable chlorophyll deficiency (Fig. 3, A) reported by Parker (7). Leaves infected with yellow stipple mosaic are almost normal in size and are only slightly roughened or distorted. Local lesions have not been produced on any variety tested thus far.

Seedlings of some varieties infected mechanically by rubbing often develop necrosis of the veins and veinlets of the inoculated leaves. The necrosis often extends into the petioles of the leaves and into the stem (Fig. 4, D) to the soil line, where it is frequently severe and causes a slight shrinking of the tissues. Necrosis has not been observed at the growing points or elsewhere above the node to which the inoculated leaves are attached. All the strains of yellow bean mosaic virus investigated by the writers produced similar symptoms; but it is not uncommon for these strains to cause necrosis of the terminal growing point on some varieties.

SUSCEPTIBILITY TESTS

Reaction of Snap and Dry Bean Varieties

Thirty-six representative varieties of snap and dry bean were inoculated with the yellow stipple virus. All varieties, including those resistant to common bean mosaic virus and its variant, proved susceptible to the virus; but in most cases the mottle symptoms were mild and the distinctive yellow stipple symptom was observed only in the dry bean varieties. Of these all except Dark Red Kidney had stem necrosis and the yellow stipple symptom. The only two snap bean varieties that had stem necrosis were pole types.

Among the snap bean varieties, mild mottle symptoms were produced on Alabama No. 1, Bountiful, Brittle Wax, Cherokee, Florida Belle, Ideal Market, Landreth Stringless Green Pod, Logan, Pencil Pod Black Wax, Plentiful, Puregold, Stringless Refugee, Sensation Refugee No. 1066, U. S. No. 5 Refugee, Sensation Green Pod, Sure Crop Wax, Tendergreen, Topcrop, and Stringless Black Valentine. In addition to a mild mottle produced on White Kentucky Wonder, stem necrosis was also noted. Varieties showing a moderate mottle were Blue Lake, Early Blue Lake, Full Measure, Giant Stringless Green Pod, Potomac, Idaho Refugee, Rival, and Unrivalled Wax. Kentucky Wonder had both stem necrosis and mild mottle.

Dry bean varieties having mild mottle, yellow stipple, and stem necrosis were Great Northern U. I. No. 123, Pinto U. I. No. 78, Red Mexican, and Robust. On Michelite and on Great Northern U. I. No. 31, the symptoms were similar except the mottling was moderate on the former and intense on the latter variety. On Dark Red Kidney, only a mild mottle was produced.

Reaction of Other Plants

The host range of yellow stipple virus was determined by inoculating 5 to 10 plants of each of 31 species, representing 20 genera in 7 families. Both local and systemic mottle symptoms are produced on a number of species in the *Leguminosae* (Table 1). The species in this family that were

TABLE 1.—*Reaction of various legumes and other species to inoculation with yellow stipple virus*

Host	Symptoms produced	
	Local[a]	Systemic
Leguminosae		
Cyamopsis tetragonoloba (L.) Taub. (guar)	+	.....
Phaseolus acutifolius Gray var. *latifolius* Freeman (tepary bean)	+	Moderate mottle and stem necrosis
Phaseolus calcaratus Roxb. (rice bean)	+	.....
Phaseolus mungo L. (urd bean)	–	Mild mottle
Pisum sativum L. (garden pea)		
var. Alderman	–	do
var. Hundredfold	–	do
var. Laxton Progress	–	do
var. Miracle	–	do
var. Perfection	–	Mild mottle and stem necrosis
var. Surprise	–	Mild vein clearing
Glycine max (L.) Merrill (soybean)		
var. Arksoy	–	Intense mottle
var. Biloxi	+	.....
var. Burdette	–	Moderate mottle
var. Cherokee	–	Very mild mottle
var. CNS	+	.....
var. Gibson	+	.....
var. Illini	+	.....
var. Lincoln	+	.....
var. Ralsoy	–	Intense mottle
Vigna sinensis (Torner) Hassk. (cowpea)		
var. Blackeye	+	Mild mottle
var. Catjang	+	do
var. Chinese Red	+	do
var. Groit	–	do
var. Iron	+	do

[a] + = infected; – = noninfected.

found resistant were *Canavalia ensiformis* (L.) D. C. (common jackbean), *Medicago sativa* L. (alfalfa var. Kansas common), *Melilotus alba* Desr. (white sweet clover), *Phaseolus aconitifolius* Jacq. (moth bean), *P. angularis* (Willd.) W. F. Wight (adzuki bean), *P. aureus* Roab. (mung bean), *P. coccineus* L. (scarlet runner bean), *P. lathyroides* L., *P. lunatus* L. (sieva Lima bean) vars. Henderson Bush and U. S. 343, *P. lunatus* var. *macrocarpus* Benth (Lima bean) vars. Fordhook 242 and Fordhook Bush, *Sesbania exaltata* (Raf.) (macrocarpa), *Trifolium incarnatum* L. (crimson clover), *T. pratense* L. (red clover), *T. repens* L. (white clover var. Ladino), *Vicia faba* L. (broad bean), and *Vigna vexillata* (L.) A. Rich.

The following species in several other families were not infected: *Beta vulgaris* L. (beet), *Cucumis sativus* L. (cucumber), *Brassica oleracea* L. (cabbage var. Early Jersey Wakefield), *Datura stramonium* L. (jimson weed), *Lycopersicon esculentum* Mill. (tomato var. Marglobe), *Nicotiana tabacum* L. (tobacco var. Turkish), *Petunia hybrida* Vilm. (petunia var. Martha Washington), *Tropaeolum majus* L. (common nasturtium), and *Apium graveolens* L. (celery).

All the varieties of *Pisum sativum*, *Glycine max*, and *Vigna sinensis*

that were inoculated were infected either locally or systemically. All systemically infected pea varieties showed only mild mottle or vein clearing, with the exception of Perfection, which also had stem necrosis. Individual soybean varieties had either local lesions (Fig. 4, A) or systemic infection, but not both. Arksoy and Ralsoy were the only two varieties with intense mottle (Fig. 4, C). All the cowpea varieties except Groit had both primary and systemic infection (Fig. 4, E). No species outside Leguminosae was infected.

SEED TRANSMISSION

Pinto U. I. No. 78, Plentiful, and Tendergreen bean plants infected with the yellow stipple virus were grown to maturity in the greenhouse. No plant among 140 grown in the greenhouse from seeds harvested from these plants developed the disease. Although this preliminary test is not conclusive, it is probable that the virus is not transmitted through bean seeds.

PHYSICAL PROPERTIES OF YELLOW STIPPLE VIRUS

Expressed juice from young infected Pinto U. I. No. 78 plants was used in these studies and inoculated to healthy Pinto U. I. No. 78 seedlings. Several trials were made in each experiment and from 8 to 12 plants were used in each trial.

Thermal Inactivation

When the yellow stipple virus was heated for 10 min. its thermal inactivation point was between 72° and 75° C. (Table 2). This range is the same as for pod mottle virus (14) but higher than for bean virus 1 and yellow bean mosaic virus, which are inactivated between 56° and 58° (8, 12). McWhorter (6) reported two strains of yellow bean mosaic that were inactivated at 60° C. The thermal inactivation point for yellow stipple virus, however, was much lower than for southern bean mosaic virus, which was inactivated between 90° and 95° C. (13).

Tolerance to Dilution

Yellow stipple virus was infectious when diluted 1 to 50,000, but not at 1 to 75,000 (Table 2). With the exception of southern bean mosaic virus, which is infectious at a dilution greater than 1 to 500,000 (13), and stipple streak virus (11), which has a dilution end point of 1 to 100,000, no other bean virus has tolerated such a high dilution. The maximum dilution at which infection by pod mottle virus occurred was 1 to 10,000 (14). Common bean mosaic virus and yellow bean mosaic virus have dilution end points at about 1 to 2,000 (8, 12).

Aging

Two methods of aging were employed: 1) Expressed juice from infected plants was held *in vitro* at 18° C.; and 2) leaves from infected plants were dried at room temperature (approximately 18° C.) for about 48 hr. and then pulverized and stored at the same temperature. In testing the

TABLE 2.—*Thermal inactivation, tolerance to dilution, and longevity of yellow stipple virus*

Thermal inactivation		Tolerance to dilution		Longevity		
Temperature (°C.)	Infection[a]	Dilution	Infection[a]	Days	In vitro	Dry tissue
					Infection	Infection[a]
Not heated	+	Not diluted	+	0	+	+
50	+	1–1000	+	1	+	0
55	+	1–5000	+	2	+	0
60	+	1–10,000	+	3	+	0
65	+	1–15,000	+	4	+	0
70	+	1–25,000	+	5	+	0
72	+	1–50,000	+	6	−	0
75	−	1–75,000	−	7	−	+
				25	0	+
				80	0	+

[a] + = infectious; − = not infectious; 0 = not inoculated.

latter material, water was added to a small portion of the dry, pulverized tissue and it was ground in a mortar, then used immediately to inoculate Pinto seedlings.

Infection was produced with the expressed juice after storage *in vitro* at 18° C. for 5 days, but not for 6 days (Table 2). This survival period is somewhat longer than that for bean virus 1 and yellow bean mosaic virus, which lose their infectivity *in vitro* after 32 hr. (8, 12). Pod mottle virus was still infectious after storage for 62 days, but not after 93 days (14), while southern bean mosaic virus was reported infectious after aging for 32 weeks (13).

Yellow stipple virus in dry leaf tissues produced infection after 80 days of storage at room temperature (Table 2). No comparison was made with the other viruses mentioned above.

SEPARATION OF YELLOW STIPPLE VIRUS FROM A MIXTURE WITH OTHER BEAN MOSAIC VIRUSES

It has previously been shown that the viruses of common bean mosaic, yellow bean mosaic, southern bean mosaic, and pod mottle can be separated from each other with little difficulty (13, 14). Yellow stipple virus can be separated from a mixture of common mosaic, its variant, pod mottle, and southern bean mosaic viruses by inoculating Great Northern U. I. No. 123 variety, because this variety is resistant to systemic infection by the above-mentioned viruses but is susceptible to the yellow stipple virus. Southern bean mosaic and pod mottle viruses produce only local lesions in this variety.

Yellow stipple virus can be separated from a mixture containing yellow bean mosaic virus by heating the virus mixture to 65° C. for 10 min. This inactivates yellow bean mosaic virus, but not yellow stipple virus. Diluting a virus mixture 1 to 5,000 inactivates yellow bean mosaic virus but not yellow stipple virus. Separation of these viruses can also be accom-

plished by inoculating cowpea which is susceptible to the yellow stipple virus but resistant to yellow bean mosaic virus.

SYMPTOMS PRODUCED BY COMBINATION OF YELLOW STIPPLE VIRUS WITH SOUTHERN BEAN MOSAIC VIRUS AND POD MOTTLE VIRUS

Since the infected bean pods from southern Illinois were more intensely mottled and malformed (Fig. 1) than pods infected with pod mottle and southern bean mosaic viruses alone, and since yellow stipple virus alone produces relatively mild symptoms on beans, it was of interest to determine whether certain combinations of these viruses upon inoculation into beans would produce more intense symptoms than the individual viruses alone. Tendergreen, Plentiful, and Stringless Black Valentine bean varieties were each inoculated with the separate viruses as well as with different combinations of the three. Each inoculum used in this test was procured from young systemically infected plants of the same variety as that to be inoculated. Since the pod mottle virus was isolated from the original pods of Stringless Black Valentine that were sent to the writers, the reaction of this variety to pod mottle virus was redetermined. The stock of Stringless Black Valentine used was systemically infected by pod mottle virus, a result which is contrary to the results of a previous investigation (14). This suggests that strains of Stringless Black Valentine may vary in their response to certain viruses.

When the yellow stipple virus was in combination with southern bean mosaic virus the symptoms produced on the three varieties were more intense than those produced by the separate viruses. Yellow stipple virus in combination with pod mottle virus, or in combination with this virus and southern bean mosaic virus, produced symptoms that were no more intense than those produced by pod mottle alone.

DISCUSSION

The yellow stipple virus is different from any bean mosaic virus heretofore described. In 1931 a condition very similar to yellow stipple was observed by the senior writer in southern Idaho on Great Northern U. I. No. 1, a variety resistant to common bean mosaic (Fig. 3, B). It was not feasible at that time to make inoculations to determine the cause of the trouble. From the similarity between these symptoms and those of yellow stipple, it appears likely that a close relationship exists. The condition noted in 1931 has not been observed in Idaho since that time.

The chlorophyll-deficiency in beans known as yellow spot and reported by Parker (7) occurred in an advanced generation selection from a cross of Stringless Green Refugee × Wells' Red Kidney, as well as in a strain of Wells' Red Kidney. Attempts to transmit this character by leaf rubbing or grafting were unsuccessful. Parker showed that yellow spot may be considered either as a simple Mendelian dominant or as being incompletely dominant to normal green. Both Stringless Green Refugee and Dark Red

Kidney, a variety closely related to Wells' Red Kidney, when inoculated with yellow stipple virus develop only a mild leaf mottling and not the yellow stippling.

The stipple streak virus of Van Der Want (11) is in no way related to yellow stipple virus. It produces local necrosis in the form of concentric rings on beans and may possibly be related to the tobacco necrosis virus.

The symptoms of soybean mosaic reported in 1921 by Gardner and Kendrick (3) and more recently by Conover (1) are similar to those produced by yellow stipple virus on soybean, but the soybean mosaic virus is not infectious to bean and its physical properties distinguish it from the yellow stipple virus.

Although the five varieties of cowpea that were inoculated with yellow stipple virus were susceptible, there appears to be no relationship between this virus, the cowpea virus reported by McLean (5), and the asparagus bean virus reported by Snyder (10). The last two viruses also are non-infectious to bean, and differ from yellow stipple virus in their physical properties.

It is of unusual interest that yellow stipple virus should have been associated in the complex with southern bean mosiac virus and pod mottle virus, two relatively new viruses of bean. According to the seed company sending the infected material, this disease complex was first noticed in the summer-planted bean crop, principally the Stringless Black Valentine variety, in Pulaski and Union Counties of southern Illinois in 1947. It was of some concern to the growers that year, but the damage was not serious. In the spring-planted crops of 1948 the disease was not sufficient to reduce yield. In the 1948 summer-planted crops, however, which were ready for market by mid-September, the disease was widespread and serious, affecting not only Stringless Black Valentine but also Plentiful and Tendergreen varieties of bean. This is the first report of widespread occurrence of the disease and of crop loss caused by combined southern bean mosaic and pod mottle viruses. They were first observed in 1940 and in 1945 in Louisiana and South Carolina, respectively, where they were of minor importance. Since pod mottle, yellow stipple, and southern bean mosaic viruses are apparently not seed-borne (although the virus of one strain of southern bean mosiac is carried in the seed to a very limited extent), it is likely that the serious outbreak of this virus complex in Illinois in 1949 may have been brought about by insect transmission from some virus-infected wild or cultivated host. A number of soybean varieties have been found to be very susceptible to these viruses when artificially inoculated in the greenhouse (13, 14). Since considerable acreages of soybeans are grown in both Union and Pulaski Counties it is possible that insects may have transmitted this virus complex from them to beans. If this was the case, it is conceivable that the soybeans did not carry so high a percentage of virus infection early in the season at the time the spring-planted snap bean crop was ready for harvest as they did later, and that therefore there was less infection than in

the summer-planted crop. By September, when the soybeans were fairly advanced in maturity, they may have had considerably more virus infection than earlier and thus offered a much larger virus reservoir for insects such as aphids to transmit the virus complex to other susceptible crops, including beans; and for that reason, widespread infection and crop losses resulted. In addition, insects feeding on infected soybeans may have migrated from these plants, because of their advanced maturity, to beans which were very green and succulent at that time.

What the economic importance of yellow stipple virus may be if it becomes widespread throughout the bean-growing areas is not known. No variety of beans has yet been found that is immune from the virus; but if in the field this virus causes symptoms no more intense than in the greenhouse, no appreciable crop loss should result. At relatively high temperatures the symptoms are considerably milder than at low temperatures. If in combination with other bean viruses yellow stipple virus produces more intense symptoms than are produced by the separate viruses, it is possible that yield might be greatly reduced.

TECHNICAL DESCRIPTION OF THE VIRUS

Marmor flavopunctum sp. nov.

Common name: Yellow stipple virus.

Specific name: From Latin *flavopunctum*, referring to the association of the species with a yellow dotting of the leaves.

Host reactions: Systemic mottle and yellow stipplelike symptoms on Pinto U. I. No. 78 and other dry bean varieties of *Phaseolus vulgaris*. Local necrotic lesions and mild systemic mottling induced in *Vigna sinensis* and *Glycine max*. Alderman, Laxton, Progress, and other varieties of *Pisum sativum* susceptible to systemic mottle infection.

Transmission: Readily transmitted by inoculation with expressed juice wiped on the leaves with carborundum. Appears not to be transmitted through bean seed. Insect vectors not known.

Physical properties: Inactivated between 72° and 75° C. in 10 min. in plant juices. Dilution end point in fresh plant extract from infected bean between 50,000× and 75,000×. Resisted aging for 5 days *in vitro* at 18° C. and more than 80 days in desiccated leaf tissue stored at room temperature.

Type locality: Southern Illinois.

SUMMARY

A new virus of bean that occurred in Stringless Black Valentine pods in combination with southern bean mosaic virus and pod mottle virus and that was separated from these viruses, is described, identified, and compared with other bean viruses. The disease caused by this virus is designated as yellow stipple. It is proposed that the virus be known as *Marmor flavopunctum* sp. nov.

Yellow stipple virus produces a mild leaf mottling. On most dry bean

varieties in addition to the mottling there are small, yellow chlorotic spots which may coalesce and form irregular patches as large as 10 mm. in diameter. Veinal and stem necrosis are frequently observed.

Of 36 bean varieties inoculated with yellow stipple virus none was resistant.

All the varieties of *Pisum sativum*, *Glycine max*, and *Vigna sinensis* that were inoculated were infected either locally or systemically. Three other species of Leguminosae (*Cyamopsis tetragonoloba*, *Phaseolus acutifolius latifolius*, and *P. coccineus*) were the only other suscepts found among 31 species representing 20 genera in 7 families.

Yellow stipple virus was inactivated between 72° and 75° C. when the juice was heated for 10 min. It was infectious at a dilution of 1 to 50,000 but not at 1 to 75,000. It resisted aging for 5 days *in vitro* at 18°. In dry tissue stored at room temperature it was still active after 80 days.

On several varieties of bean, yellow stipple virus in combination with southern bean mosaic virus produced symptoms more intense than those produced by the separate viruses. In combination with pod mottle virus, as well as with this virus and southern bean mosaic virus, the symptoms produced were no more intense than those of pod mottle alone.

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FACTORS INFLUENCING INVASION OF POTATO BY ERWINIA CAROTOVORA[1]

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INTRODUCTION

In the summer of 1947 the authors conducted some preliminary tests on eight varieties of potatoes with the thought that if susceptibility to soft rot was increased by exposure to heat, as reported by Nielsen (1), Nielsen and Todd (2), and Rose and Schomer (3), there might be significant differences in the reactions of different varieties to this predisposing factor. Using the methods described by Nielsen (2), slices of freshly harvested tubers were inoculated with runoff water from a commercial potato washer after exposure to solar heat for 2 hr., which raised the temperature of the tuber tissue at a depth of 10 mm. to 46°–48° C. The rots which developed, in much the manner described by Nielsen (1), were not attributable to *Erwinia carotovora* (Jones) Holland on the basis of isolations. There were some differences in the percentage of infection and extent of rot with different varieties, but these differences were relatively small. The varieties had been harvested at the same time and therefore at different stages of maturity. The results indicated the necessity of growing the potatoes in the greenhouse where they could be harvested and tested at comparable stages of maturity and where exposures to heat could be controlled and inoculations made with pure cultures of bacteria.

Other preliminary tests were carried out in the laboratory using the same technique with a culture of *E. carotovora* and greenhouse-grown tubers of Bliss Triumph, Red Warba, and Pawnee varieties. Tubers treated under infrared lamps were compared with untreated controls. In this experiment, as in field tests, the temperature of treated tissue was approximately 47° C., determined by means of a thermometer inserted into the tuber to a depth of 10 mm. While this temperature is slightly above the sublethal maximum determined by Nielsen (1), it was considered essential because the slicing technique involves the testing of tissue located at the base of the thermometer bulb. This method failed to produce any consistent differences due to varietal reaction or to the effect of the heat treatment on the area of the inoculated surface showing infection. Contrary to expectations, however, the depth of penetration was 40 per cent greater in the unheated tubers than in those exposed to heat.

[1] These studies were conducted at the Nebraska and the Connecticut Agricultural Experiment Stations and are published with the approval of the Directors; Paper No. 483, Journal Series, Nebraska Agricultural Experiment Station.

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The following experiments were then undertaken to study the effects of heat, water, stages of maturity, and time of holding upon the reaction of 11 varieties of freshly harvested potato tubers to a single isolate of *E. carotovora*. The results are meaningful in that they differ from those previously reported by other workers using soft-rot bacteria with high optimum temperatures as the test organism (1, 2), and because they furnish an explanation for the success of certain practices which have been recommended for the control of bacterial soft rot (4, 5).

MATERIALS AND METHODS

The work was initiated and carried on for a year at Nebraska[4] and then continued in Connecticut. The potato varieties used and the methods employed varied somewhat at the two locations and are therefore outlined under the experiments. The following methods and materials were used uniformly in all experiments at both locations, unless otherwise noted.

The tubers, immediately after digging, were brought into the laboratory, washed in tap water, and placed in 60 per cent alcohol for about 1 min. to partially disinfect them. They were then allowed to surface-dry before being subjected to any of the experimental conditions. The various heat treatments were all designed to raise the temperature of the outer 10 mm. of tuber tissue to approximately 42° C. as determined with a thermometer inserted to this depth. This temperature with less than a 35-min. exposure to infrared lamps was found to be sublethal in preliminary tests upon 10 varieties. Exposure of 25 to 35 min. raised the temperature to 40° to 43.5° C. A 30-min. exposure was usually employed.

Six tubers of each variety were subjected to each particular treatment and then left on the laboratory bench until heat and surface moisture had been dissipated before being placed in sterilized moist chambers. All single tubers representing a single treatment were placed in the same chamber and held for 1 hr. before inoculation.

An American Type Culture Collection strain of *E. carotovora* was used in all experiments. The concentration of inoculum washed from a 24-hr. slant culture of the organism growing upon Wernham's buffered potato-dextrose agar was standardized by the loop dilution method employing a 4-mm. loop of 24 B. and S. gauge wire submerged to a depth of 9.5 cm. in an 85-ml. test tube of suspension.

Inoculation was made by holding a 4-mm. loop of this dilute suspension to the treated, dry surface of a tuber and pushing through the inoculum a dissecting needle, bent twice so as to form a stop, into the tissue of the tuber to a depth of 2 mm. Each tuber was inoculated at three points on the upper surface in this manner. Tubers were incubated in the moist chambers at room temperatures ranging from 23° to 26° C., for 48 hr. At the termination of this period each rot lesion was hollowed out with a spatula of appropriate size and the diameter and depth of the rotted tissue determined.

[4] This work was aided in part by a grant from the Nebraska Potato Development Commission.

Diameter and depth values were averaged for each tuber and the average values used to calculate the lesion volume, represented as the volume of a cylinder (π r^2 d). Accordingly, six cylinder volume values, one for each set of three inoculations per tuber, were calculated for each variety and treatment test. The means of these six values are in tables 1 and 2; in tests upon the Katahdin variety the separate values are given in tables 4 and 5.

THE EFFECT OF DIFFERENT TREATMENTS UPON SUBSEQUENT INVASION OF POTATO

Ten varieties of potato were grown in the greenhouse and two varieties at a time were harvested and tested as they matured. It was possible to test only six tubers of each of two varieties simultaneously and the tests therefore extended over a period of 24 days. As a result not all of the tubers were at exactly the same stage of maturity, but all vines were mature and the soil around the tubers was kept moist until they were harvested.

In the first experiment, six tubers of each of 10 varieties were inoculated with *E. carotovora* after various treatments to determine the effect of heating the tubers on subsequent invasion.

The heat treatment consisted of exposing the tubers under three Fisher No. 11–504–30 infrared lamps arranged so that the bulbs were approximately 15 in. above the bench and directed toward a common center to give a core of dim illumination surrounded by a circle of relatively intense light. Tubers were placed in the central area for treatment because it was found that this area was of fairly uniform temperature throughout. One lot of tubers was exposed under the infrared lamps until the tuber tissue, at a depth of 10 mm., registered 42° C. These were all inoculated after division into three lots; one lot was cooled in air, another in water, and a third was held at room temperature for 24 hr. A fourth lot of tubers was inoculated immediately after harvesting without heating, and the fifth lot was held 24 hr. after harvest and then inoculated.

The results (Table 1) reveal considerable variability among mean infection values, but it is obvious that heat treatment of the tubers followed by cooling in air reduced invasion considerably below that found in the unheated controls. Holding the tubers at room temperature for 24 hr. after heating further reduced invasion to the lowest point of any of the treatments. The heated tubers cooled in water before inoculation, on the other hand, showed slightly more invasion than the unheated controls even when inoculated immediately after digging.

It will be seen that the range of values in table 1 represents discontinuous variation. This will be better understood if we examine, in tables 4 and 5, the volume values for lesions upon individual tubers of the Katahdin variety. Values may range from 0 to more than 400 cu. mm. within a single treatment. Obviously, certain conditions must be met before

TABLE 1.—*Reaction of 10 varieties of potatoes to Erwinia carotovora as modified by five different treatments. Each value represents the average calculated cylinder volume of three rot lesions on each of six tubers, expressed as cubic millimeters*

Variety[a]	Heated by infrared lamps to 42° C. at 10-mm. depth and inoculated after:			Unheated controls inoculated		Geometric means
	Cooling in		Holding 24 hr.	Immediately after digging	24 hr. after digging	
	Air	Water				
Red Warba	53.0	1375.7	6.5	832.2	854.2	7.1
Bliss Triumph	0.0	0.0	24.0	114.7	1365.2	3.8
Cayuga	1562.2	1842.8	329.0	890.5	612.2	11.5
Teton	318.3	991.7	418.3	1574.2	164.0	9.8
Pawnee	160.0	1456.5	0.0	1068.2	791.8	8.6
Kasota	1.2	1869.5	46.8	980.3	502.5	7.5
Irish Cobbler	263.8	159.5	23.5	607.7	220.0	6.4
Desota	0.0	173.7	20.2	248.2	1815.2	5.7
Nebr. No. 2	10.7	306.5	0.0	702.3	0.0	3.4
Katahdin	47.7	36.8	0.0	200.0	0.0	1.9
Arithmetic means	241.7	821.3	86.8	721.8	632.5	
Geometric means	4.3	8.4	2.5	9.7	8.0	

[a] The last eight varieties are arranged in decreasing order of susceptibility to invasion as indicated by geometric means. Compare with the same eight varieties in table 2.

infection can occur and the 48-hr. period, after inoculation and before the results are recorded, operates to magnify the difference between infected and noninfected tubers. These data show that, as far as infection is concerned, the tubers might be divided into two classes, infected and noninfected. The latter class of tubers is characterized by a fixed value, whereas values for infected tubers increase continuously with time. It is this departure which results in discontinuity.

In order to render the data amenable to an analysis of variance, the original tuber unit-volume values, each represented by x, were transformed to log $(0.1\ x + 1)$. Averages of these transformed values are represented by the geometric means. These means were compared in the statistical analyses and are included in both tables 1 and 2 in order to show the variation among varieties and treatments[5].

When the experiment was repeated using only eight varieties, the results were similar to those reported in the first experiment (Table 2). This experiment, using all 10 varieties, was conducted from April 5 to 29; and the second experiment, with only eight varieties, on May 12 to 18. The mean time between tests for any one variety was approximately 28 days. A comparison of volume values in tables 1 and 2 reveals that the invasion of tuber tissue was less in the second experiment. Part of this difference may result from changes in the tubers associated with maturity. All plants were mature when dug and those which could not be used im-

[5] The writers are indebted to Dr. C. I. Bliss for his aid in directing the statistical analyses.

TABLE 2.—*Reaction of eight varieties of potatoes to Erwinia carotovora as modified by five different treatments. Each value represents the average calculated cylinder volume of three rot lesions on each of six tubers, expressed as cubic millimeters*

Variety[a]	Heated by infrared lamps to 42° C. at 10-mm. depth and inoculated after			Unheated controls inoculated		Geometric means
	Cooling in		Holding 24 hr.	Immediately after digging	24 hr. after digging	
	Air	Water				
Cayuga	17.3	1330.8	0.0	1300.2	610.8	7.0
Teton	68.2	1805.2	14.2	1096.2	392.7	6.9
Irish Cobbler	1.3	752.7	6.8	1183.7	290.5	4.9
Pawnee	23.5	3.0[b]	385.2	691.2	535.8	4.8
Kasota	4.2	718.3	1.2	268.0	231.0	2.8
Katahdin	604.3	144.0[b]	0.0	1.2	0.0	2.0
Desota	138.2	0.0[b]	0.0	591.0	176.8	1.9
Nebr. No. 2	41.8	18.7[b]	0.0	652.3	4.7	1.9
Arithmetic means	112.4	596.6	50.9	723.0	280.3	
Geometric means	2.0	6.3	0.9	6.7	4.4	

[a] Eight varieties arranged in decreasing order of susceptibility to invasion as indicated by geometric means.

[b] Cooled in a refrigerator at 5° C. instead of in water.

mediately were left in the soil, which was kept moist until the tubers could be used.

The only departure of the results in table 2 from those in table 1 was in the data for the four varieties that were cooled at 5° C. in the refrigerator instead of in cold water. These four varieties had less instead of more invasion as compared with those cooled in air. The average of the first four varieties cooled in water was much higher than that of the varieties cooled in air, as in experiment 1, and was much higher than in the unheated tubers. These differences were probably due to the effect of drying rather than heating the tubers, as will be pointed out in later experiments.

The results of analyses of the separate experiments are not given;

TABLE 3.—*Analysis of variance of data in tables 1 and 2 combined: Reaction of eight varieties of potatoes to Erwinia carotovora as modified by five different treatments (two tests)*

Source of variation	D.F.	M.S.	F.
Among varieties	7	67.4614	6.92**
Varieties × experiments	7	7.1728	0.74
Heat treatment	1	321.8885	33.01**
Holding 24 hr.	1	67.8358	6.96*
Treatment × holding	1	3.8858	0.40
Water vs. air cooling	1	138.2369	14.18**
Treatments × experiments	4	1.1506	0.12
Error	56	9.7512	1.00

* Probability less than 5 per cent.

** Probability less than 1 per cent.

however, pertinent facts brought out by analysis of the combined experiments treating the eight varieties common to both (Table 3) show that the two experiments are entirely consistent with one another and might be considered as one experiment. Four individual comparisons, represented each by a single degree of freedom, were segregated for special study in the first experiment. The interactions of these individual comparisons with varieties produced nonsignificant mean squares of similar magnitude which are not given, but it may be stated that all of the varieties reacted qualitatively toward the treatments as if they had comprised a single variety and that the order of these interactions was of the same magnitude for all treatments. In the combined analysis the interactions of these four individual comparisons with experiments produced nonsignificant mean squares. As an example of these values, not shown, (water vs. air cooling) × experiments yielded a mean square of 0.13.

RELATIVE SUSCEPTIBILITY OF VARIETIES TO INVASION

Varietal reaction to invasion was independent of both treatments and experiments and differences in susceptibility of the varieties to invasion were highly significant (Table 3). It can be seen by comparing the orders of susceptibility to invasion of the varieties in the two experiments that on the basis of geometric means they were entirely consistent in their reaction between experiments. Indeed, if the average susceptibility of each variety is determined by the position in both tables 1 and 2 they will be found to hold the order given in table 1.

THE EFFECT OF TISSUE DRYING UPON INVASION

The results of the first two experiments indicated that tissue drying might be the factor involved in the decreased invasion of heated tubers after cooling in air as contrasted with cooling in water. Further experiments were therefore made to delineate more clearly the effect of drying on the invasion of tuber tissue.

Katahdin potatoes harvested in October were immediately brought into the laboratory and placed in warm water at 40° C. for about 10 min. until room temperature (23.5° C.) was indicated on a thermometer inserted 10 mm. into the tuber. They were then either subjected to heat treatment under infrared lamps, as in previous experiments, or placed in a water bath fitted with a mechanical stirrer and maintained at 50° to 60° C. for 10 to 15 min. or until the temperature of the tuber tissue reached the desired point. Following the heat treatments each lot was divided and one-half (six tubers) cooled to room temperature in water and the other half in air. Six unheated tubers were similarly inoculated and used as controls.

The results (Table 4) clearly indicate that drying is probably the major factor in the decreased invasion following the heat treatments found in experiments 1 and 2. The only treatment in this experiment resulting in less invasion than that occurring in the unheated controls was the heating

TABLE 4.—*The effect of heat and moisture upon reaction of Katahdin potatoes to Erwinia carotovora. Each value represents the average calculated cylinder volume of three rot lesions on individual tubers, expressed as cubic millimeters*

	Heated by infrared lamps to 42° C. at 10-mm. depth and inoculated after		Heated in water to 42° C. at 10-mm. depth and inoculated after		Unheated inoculated controls
	Cooling in		Cooling in		
	Air	Water	Air	Water	
	0.00	431.75	408.45	494.55	37.68
	0.00	179.86	45.15	565.20	21.20
	6.28	467.48	84.78	314.00	37.68
	6.28	4.90	160.77	431.75	126.95
	12.56	0.00	58.88	565.20	21.20
	0.00	325.12	8.48	271.55	3.14
Means	4.19	234.85	127.75	440.38	41.31

under infrared lamps followed by cooling in air. This treatment probably resulted in more drying of the tissues than any of the other treatments. Even when heated in water those tubers cooled in air were not so extensively invaded as when cooled in water, although all tubers heated in water had more invasion than the unheated controls. This effect might be attributed to increased sugar permeability of heat-treated tissues as determined by Nielsen and Todd (2), plus the presence of sufficient water for the growth of *E. carotovora* and for the mobility of its enzymes.

The effect of tissue drying was studied further by means of additional treatments consisting of heating one lot of tubers in an electric oven for about 1 hr. at 50° C. until the tuber tissue reached 42° C., and using both dry and presoaked unheated tubers as controls.

The results (Table 5) are in general agreement with those of table 4 and further confirm the hypothesis that drying is a major factor in decreasing susceptibility to invasion. No infection was obtained when tubers were

TABLE 5.—*The effect of drying of cortical tissue upon reaction of Katahdin potatoes to Erwinia carotovora. Each value represents the average calculated cylinder volume of three rot lesions on individual tubers, expressed as cubic millimeters*

	Inoculated after heating to 42° C. at 10-mm. depth by						Unheated and inoculated	
	Lamp		Oven		Water			
	Air-cooled	Water-cooled	Air-cooled	Water-cooled	Air-cooled	Water-cooled	Unsoaked	Presoaked
	0.00	7.07	0.00	0.00	0.00	332.46	4.18	0.00
	0.00	0.00	0.00	0.00	0.00	284.36	192.33	12.56
	0.00	2.07	0.00	21.20	7.07	251.20	190.00	9.42
	0.00	0.00	0.00	192.33	6.28	266.27	141.30	9.42
	0.00	7.07	0.00	192.33	175.84	301.44	301.44	141.30
	0.00	392.50	0.00	340.25	84.78	192.33	251.20	203.88
	0.00	283.54	0.00	58.87	84.78	192.33	251.20	301.44
	0.00	31.40	0.00	251.20	175.84	113.04	251.20	79.44
Means	0.00	90.46	0.00	132.02	66.82	241.68	197.86	94.68

heated and cooled under dry conditions. Heating in water and cooling in air gave about the same results as heating dry and cooling in water. The greatest invasion occurred when tubers were heated in water and cooled in water.

THE EFFECT OF DEPTH OF INOCULATION UPON INVASION

The effect of heating and cooling was determined in the previous experiments solely on the basis of invasion of the peripheral tissues, inasmuch as inoculations were made at a uniform depth of 2 mm. Whether the treatments exerted an influence on the deeper tissues could be determined by comparing the effects of inoculations made at different depths, and such an experiment was therefore set up. Tubers heated to 42° C. under infrared lamps were cooled in various ways and inoculated at two different depths, 2 mm. and 4.5 mm.

As would be expected, a greater extent of invasion occurred with the deeper inoculation (Table 6). The least invasion with both methods of

TABLE 6.—*Invasion of Teton potatoes as modified by depth of inoculation. Each value represents the average cylinder volume of 18 rot lesions on six tubers, expressed as cubic millimeters*

Replications, 6 tubers each	Heated by infrared lamps to 42° C. at a depth of 10 mm. and inoculated after					
	Cooling in air		Cooling at 5° C. in refrigerator		Cooling in water	
	Inoc. depth 2 mm.	Inoc. depth 4.5 mm.	Inoc. depth 2 mm.	Inoc. depth 4.5 mm.	Inoc. depth 2 mm.	Inoc. depth 4.5 mm.
1	119.10	82.60	180.78	499.66	201.19	650.59
2	122.39	227.25	179.33	455.40	232.89	436.83
3	135.73	484.08	187.33	615.12	150.79	622.18
Means	125.73	264.64	182.48	523.39	194.96	569.87

inoculation occurred, as in previous experiments, in those tubers cooled in air as contrasted with those cooled in water. Those cooled in the refrigerator at 5° C. showed more invasion than those cooled in air, whereas the reverse was true with the four varieties cooled in the refrigerator in a previous experiment (Table 2, footnote 3). The difference here may well be due to differences in the two refrigerators used (5). In this latter case the refrigerator used for incubation of inoculated tubers was equipped with a humidifying device consisting of a fan arranged to circulate air over iced coils. The continuous evaporation of moisture from the coils probably resulted in a higher humidity than in the refrigerator used in the second experiment.

DISCUSSION

In contrasting the results of these experiments with those of Nielsen (1), Nielsen and Todd (2), and Rose and Schomer (3), it should be

pointed out that these experiments were conducted with a single strain of *E. carotovora*, whereas the reports cited above dealt with soft rots in a general sense without identifying the pathogens or identifying them (2) as thermophilic bacteria and not *E. carotovora*. The results herein reported supply an explanation of the control of soft rot caused by *E. carotovora* through the practice recommended by Ruehle (4) of drying washed potatoes in hot air.

SUMMARY

Heating freshly harvested tubers under infrared lamps until the tissue reached a temperature of 42° C. at a depth of 10 mm. did not predispose tubers to invasion by *E. carotovora*.

When such heated tubers were cooled in air to room temperature before inoculation the amount of rotting caused by *E. carotovora* was less than in unheated tubers.

When tubers were heated in water to the same temperature (42° C.) the amount of rot was greater than in the unheated controls and when such heated tubers were cooled in air the rotting was less than when cooled in water.

The amount of rot was greater when the inoculations were made at a depth of 4.5 mm. as contrasted with 2 mm., but the relative effect of cooling in air after heating was similar at both depths of inoculation.

Varietal differences in susceptibility to invasion were found to be highly significant.

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REPORT AND ABSTRACTS OF THE SEVENTH ANNUAL MEETING OF THE POTOMAC DIVISION OF THE AMERICAN PHYTOPATHOLOGICAL SOCIETY

The seventh annual meeting of the Potomac Division of The American Phytopathological Society was held in the Plant Industry Station Auditorium, Beltsville, Maryland, March 9 and 10, 1950. The attendance was good, varying from 75 to 150 at the different sessions. One hundred and three enjoyed the banquet and social feature at the close of the first day's formal program, which was followed by an excellent address by Dr. E. C. Stakman on "Science in Human Affairs." Twenty-eight formal papers were presented. Two new features were initiated at this meeting: 1) A short informal discussion of new pathological problems, and 2) a demonstration of some lines of work in progress at the BPI Station.

Officers elected to serve through the 1951 meeting:

President, C. E. Cox; *Vice-President,* S. P. Doolittle; *Secretary-Treasurer,* J. B. Demaree; *Councilor,* C. L. Lefebvre.

J. B. Demaree, *Secretary-Treasurer*

ABSTRACTS OF PAPERS PRESENTED AT THE MEETINGS

Some Host Plants of Chrysanthemum Stunt Virus. Brierley, Philip. Producers of florist's chrysanthemums (*Chrysanthemum hortorum*) have been remarkably successful in reselecting healthy stock since stunt was recognized as a virus disease. Nevertheless, practical control and other studies have been hampered by the lack of a quick test for presence of the virus. Test varieties of chrysanthemum selected by Olson and by Keller react after 2 to 3 months. Manual inoculation of 30 Compositae, including eight species of Chrysanthemum and species from 21 other genera, has thus far revealed three additional plants that expressed symptoms, and nine plants—*Chrysanthemum carinatum, C. coronarium* (received as *C. segetum*), *Achillea ptarmica, Athemis tinctoria, Centaurea cyanus, Dahlia variabilis, Echinacea purpurea, Emilia sagittata,* and *Liatris spicata* —that developed no recognizable symptoms but from which the stunt virus was recovered in the florist's chrysanthemum Mary MacArthur. *Chrysanthemum praealtum* (received as *Pyrethrum parthenifolium*) expressed marked stunting with smaller, paler leaves and smaller flowers. *C. coccineum* (*P. roseum*) showed slight stunting. The florist's cineraria (*Senecio cruentus*) developed primary spots 20 to 30 days after inoculation, followed by upright growth with paler leaves ruffled at the margins. Cineraria promises to be useful as a test plant for the chrysanthemum stunt virus.

Spines of Rust Spores. Cassell, R. C., Helen L. Woods, and Frances Meehan. Outer surfaces of the uredospores of ten rusts found on graminaceous hosts were examined at magnifications of 13,000 to 92,000× with an electron microscope. The spiny projections from the spores are similar in most of the rusts studied. Variations in form and arrangement of the spines are frequently as great among the spores of a single rust, as among the spores of the various species and varieties. Spores, irrespective of age or origin, are covered with the same type and size of awl-shaped spines. The spines appear to be a continuation of the epidermis of the spore, blending smoothly into the outer part of the body. Immature spores have spines of irregular arrangement, or occasional partial alignments on the surface, whereas mature spores commonly have spines in fairly definite patterns or rows. The spines average about $\frac{1}{4}$ μ in diameter at the base, and between $\frac{1}{3}$ and $\frac{1}{2}$ μ in length. A mature uredospore of wheat stem rust (*Puccinia graminis tritici*) measuring 20×32 μ has at least 200 to 300 spines and probably more.

Spoilage of Sweet Potatoes Stored at 50°, 55°, and 60° F. Cooley, J. S., and Helen F. Smart. The sweet potato, a plant of tropical origin, is extremely sensitive to continuous storage at even moderately low temperatures. The temperature usually considered proper for sweet-potato storage is about 55° F. This study involves a comparison of the quantity and kind of spoilage occurring at 55° and also at temperatures 5° below and 5° above this possible optimum. More spoilage occurred in all varieties

tested when stored at 50° than at 55° or 60°, which possibly indicates that 50° is a "chilling" temperature for sweet potatoes. Physiological breakdown became more prevalent at 50° F. as the sweet-potato storage season advanced. Potatoes that had been weakened in this way by continuous storage at a chilling temperature of 50° were readily rotted by relatively weak pathogens. The dividing line is often very narrow between breakdown resulting from some unfavorable environment and the spoilage resulting from some pathogen attacking a weakened potato. Some varieties of sweet potatoes are less tolerant than others of continuous storage at 50°. Nancy Hall, the Jersey types, and yellow strains of the latter showed greater spoilage at 50° than did Porto Rico.

Internal Browning of Tomatoes in Maryland. COX, C. E., AND L. O. WEAVER. Internal browning of tomatoes has been recognized in this and neighboring States since 1945. It was found in five counties in Maryland in 1949. Losses have been small due to limited occurrence of infected plants and to the tendency for diseased plants to produce many normal fruits. There is apparently no difference in varietal susceptibility among Rutgers, Marglobe, Garden State, Southland, Pan America, Ontario, Clower's Globe, Clinton Hybrid F2, and five breeding lines, all of which have been found infected. Cuttings from diseased plants in the field were approach-grafted to healthy tomato and tobacco plants in the greenhouse on August 14. Within 10 days grafted tobacco had mosaic symptoms. The tomatoes had mild, interveinal mottling at the base of young leaflets. Some fruits that ripened between November 13 and December 12 had typical internal browning. All fruits ripening thereafter were symptomless. Juice from infected tomato plants applied with carborundum resulted in mild mottling of tomato foliage, mosaic and stunting of tobacco, necrotic spotting of *Nicotiana glutinosa*, flecking of primary leaves of cucumber, and bleaching of leaf bases accompanied by severe stunting and defoliation of pepper. In electron micrographs, purified virus from *N. glutinosa* could not be distinguished from tobacco mosaic virus.

The Development of Urnula craterium on Old Strumella-Infected Hardwood Stems. DAVIDSON, ROSS W. In areas where *Strumella coryneoidea* is common on oaks and other hardwoods, *Urnula craterium* was found developing in abundance on old cankered stems that had been lying on the damp soil for several years. *Urnula craterium* began developing in the fall during damp cool weather (October or November) and continued to develop during warm spells throughout the winter. The fingerlike fruiting primordia gradually swelled at the tip and broke open during warm weather in late March and April, but in 1949 mature ascospores were not found in sporophores before April 25. In late April and early May discharged ascospores germinated readily on ordinary culture media, whereas conidia of *Strumella* are not known to be germinable on such media. Cultures from ascospores of *Urnula craterium* are very similar to cultures from Strumella cankers. The possibility that *Urnula craterium* is the perfect stage of *Strumella coryneoidea* suggests that infected trees should not be cut and left on the ground in the stand. It is believed that this fungus commonly grows as a saprophyte on dead or dying hardwoods and that the perfect stage will develop after such stems have been lying on damp soil for some time.

Fragaria vesca as an Index Plant for Some Virus Diseases of Strawberry. DEMAREE, J. B. The wild strawberry of Europe, *Fragaria vesca* L., has been proved by investigators in the British Isles to be extremely sensitive to some forms of strawberry viruses. A clone secured from the East Malling Research Station, Kent, England, has been given preliminary trials at the Plant Industry Station, Beltsville, Maryland. These tests have shown that *F. vesca* is a much more suitable indicator plant for the strawberry yellows than the American variety, Marshall, heretofore used. Diagnostic symptoms in *F. vesca* when infected with several collections of virus-infected plants were clear and definite, especially when the virus was transmitted through stolon grafts. Symptoms were extreme dwarfing, thin short petioles, and very small leaves. Infected plants of the variety Marshall obtained from Oregon induced faint mottling in addition to dwarfing in the index plants. Most varieties collected from the East and grafted on *F. vesca* caused the petioles to recurve in absence of leaf mottling, although two eastern collections induced extreme leaf distortion and mottling. When the eastern species of the aphid vector, *Capitophorus* sp., was used to transmit the virus most common in the East, the symptoms were somewhat different, the dwarfing was less extreme, petioles were moderately shortened, and the leaves were distorted, crinkled, and slightly mottled. These preliminary trials confirm the conclusions of Harold E. Thomas, that there may be a complexity of strawberry virus entities in the United States.

Transmission of Downy Mildew of Cucumber by the Spotted Cucumber Beetle. DOOLITTLE, S. P., AND F. S. BEECHER. In the summer of 1949 spotted cucumber beetles, *Diabrotica undecimpunctata howardi*, were fed for 24 hr. on cucumber leaves showing numerous sporulating lesions of downy mildew, *Pseudoperonospora cubensis*. The insects

then were allowed to move into glass containers and transferred to healthy cucumber plants in cages in the field. Twenty-five beetles were placed in each of five cages containing three cucumber plants with four to five leaves. Fifteen days later all of the 15 plants in cages containing beetles had lesions of downy mildew. Fifteen plants in five adjacent cages containing no beetles remained healthy. On August 23, cucumber plants grown in the greenhouse were transplanted under cages in the field in lots of three per cage. On August 26, beetles were collected in a field of cucumbers where mildew was prevalent and transferred at once to five of the cages in lots of 30 insects per cage. On September 13 downy mildew was present on seven of the 15 plants in the cages where beetles were present. No mildew had appeared by September 26 in the five cages of plants containing no beetles. These data indicate that the spotted cucumber beetle is a factor in the dissemination of the downy-mildew fungus.

Airplane Survey for Pole Blight of Western White Pine. FOWLER, MARVIN E. Pole-size western white pine, *Pinus monticola*, is being attacked by a severe killing disease of unknown cause. The disease was first observed 20 years ago by a forester in northern Idaho and is now known in British Columbia and northern Idaho and adjacent Montana and Washington. A survey was initiated by the Division of Forest Pathology, U. S. Department of Agriculture, in the summer of 1949 to determine the southern extent of the disease and to obtain information on the intensity of the disease and its rate of spread. Because of the characteristic form of white pine and the yellowing of needles and shortening of needle length and terminal shoot growth of diseased trees, airplanes proved to be very satisfactory for scouting. In small planes flown about 500 ft. above the tree tops, pathologists were able to spot disease-suspected stands. Ground crews then examined suspected trees to determine whether the characteristic necrotic lesions were present on the main stems. Infected stands were found in the St. Joe area of Idaho, only 50 miles north of the famous Clearwater timber stands where 70 per cent of the western white pine is cut.

Wetwood in Living Trees. HARTLEY, CARL, AND ROSS W. DAVIDSON. Attention was called in 1937 to excessive wetness in the trunk wood of living trees, which was not traceable to direct entrance of rainwater. Particularly in broadleaved species this wetwood condition is commonly associated with relatively high pH, fermentation odors, and gas pressure, and with bacterial infection which is regarded as the most probable cause. Willows, true poplars, and elms usually contain such wetwood. Since the earlier report, wetwood has been observed in one additional species each of oak and hemlock, two more of the true firs, in apple and tulip poplar, and less typically in birches and maples. In noble fir as in the broadleaves the pH of the outer wetwood has been higher than that of the normal sapwood. Further studies are needed also on the relation of wetwood to frost cracks and slime flux, to the premature mortality of Lombardy poplar and other species, to the toughness and mineral content of the wood, and to some of the defects that develop during the seasoning of lumber. The confusion in some genera as to the differentiation of sapwood and heartwood could probably be lessened by considering wetwood-infected and uninfected trees separately.

Relation of the State Extension Plant Pathologist to His Subject-Matter Department. HASKELL, R. J. The results of a questionnaire dealing with the relationships of extension plant pathologists to the research and teaching personnel and to the heads of their departments is given. Thirty-eight questionnaires were sent out and 32 replies were received. Twenty out of 32 specialists replying devote part time to other phases of work such as research and teaching. Twenty-eight are officed in their department. Twenty-five hold academic ratings in the college, seven do not. In most cases the department head is responsible for the subject matter taught. In twenty-five cases the head is concerned with the specialist's appointment.

Studies on Competition among Physiologic Races of the Leaf Rust of Wheat. IRISH, KENT R. Preliminary studies indicate that competition is manifest between closely associated races growing in composite culture. Four important races of leaf rust, 9, 15, 58, and 126, were mixed in equal amounts and grown together on the uniformly susceptible host variety Cheyenne. A decline and/or disappearance of a race from the association was considered to be evidence of competition between one or all of the races in the mixture. Composite culture I was carried through seven generations and composite culture II was continued through 10 generations. The race populations in each generation were sampled by making series of single-pustule isolations. The isolates were cultured and the races identified. One hundred and three isolations were made from the generations of culture I and 158 from those of culture II. Physiologic races 9 and 15 were found to be the dominant competitors when grown with races 58 and 126. The latter two races were unable to maintain themselves in the mixed cultures and were eliminated in the earliest cultural generations. The results of both experiments indicate that

race 58 is the weakest competitor of the four races tested. Race 15 was somewhat the best competitor in tests with composite culture I, whereas race 9 was able to maintain itself in almost equal abundance. Race 9 was by far the best competitor in the second experiment as race 15 disappeared from the culture by the ninth generation.

Pathogenicity of Physiologic Races of Erysiphe graminis tritici in the Southeastern United States. LOWTHER, C. V. Powdery mildew of wheat (*Erysiphe graminis* DC.) has increased steadily in the Southeastern United States in recent years. Varieties of wheat that previously had been resistant to mildew became severely infected, indicating that new races were present in the area. Forty collections of mildew obtained in 1948 and 1949 were identified as to race. Nine distinct races were differentiated on the varieties Axminster C.I. 8195, Ulka C.I. 11478, Chul C.I. 2227, Hope C.I. 8178, and Normandie C.I. 12747. Eight of the races were collected within a radius of 70 miles of Beltsville, Maryland; the other one came from near Winston-Salem, North Carolina. All the commercial varieties of wheat grown in the Southeast are susceptible to one or more of these nine races of mildew, but some varieties have more tolerance than others. A few noncommercial wheats, that have a high type of resistance to all the races thus far identified, are available for use in breeding.

Effect of Temperature on the Development of Some Diseases of Gladiolus, Narcissus, and Lilium. MCCLELLAN, W. D., AND B. H. MARSHALL, JR. Optimum temperatures for the growth of *Botrytis gladiolorum*, *Curvularia lunata*, and *Stemphylium* sp., all from gladiolus, in culture were: 65°–75°, 75°–85°, and 65°–75° F., respectively. Infection of Picardy gladiolus foliage with *B. gladiolorum* occurred at temperatures of 35° to 75°, with the optimum between 55° and 65°. Infection with *Curvularia* occurred at 45° to 85°, with the optimum between 75° and 85°. Infection of foliage at temperatures higher than 85° was not tested. The optimum temperatures for growth of *Botrytis elliptica* and for *Colletotrichum lilii* were 65°–75° and 75°–85°, respectively. Infection of Easter lily foliage with *Botrytis* took place at 35° to 75° but not at 85°. Infection took place more slowly but lesions were numerous and small at the lower temperatures, whereas the disease was most severe at 65°. The optimum temperature for growth of *Fusarium oxysporum* f. *narcissi* was 75°. King Alfred narcissus bulbs inoculated and grown at soil temperatures of 45°, 50°, 55°, 60°, 65°, and 70°, became infected most rapidly at 65° and 70°. Foliage symptoms were delayed 3 weeks when the soil temperature was 55° as compared with 60°. Optimum growth of uninoculated bulbs occurred at 55° to 60°. Inoculated bulbs flowered normally at 55° before foliage symptoms became evident.

Mercury Content of Narcissus in Relation to Plant Injury. MCCLELLAN, W. D., ALSTON W. SPECHT, B. H. MARSHALL, JR., JOHN W. RESNICKY, AND C. J. GOULD. Narcissus bulbs were treated in Washington, North Carolina, Long Island, and Maryland with New Improved Ceresan, Ceresan M, 2 per cent Ceresan, Mersolite 8, Spergon, or Arasan for the control of Fusarium basal rot. Similar lots were untreated. Many of the plants from bulbs treated with the Ceresans were injured. Most severe injury occurred with King Alfred bulbs treated with the Ceresans at Long Island. Most of these bulbs produced normal foliage, but some developed yellow leaves and the foliage of others made very little growth. The centers of a few bulbs were entirely rotted. Spectrochemical analysis showed the mercury content of these bulbs to be correlated with the degree of injury. Mercury content was greatest in the centers and basal plates of the bulbs and in the shoots. It was least in the outer scales. Mercury content in the center of these bulbs was as follows: bulbs with centers decayed, 300 p.p.m.; bulbs producing very little foliage, 125 p.p.m.; and bulbs producing yellow foliage, 75 p.p.m. None was detectable in the untreated. Bulbs from the other sources had little foliage injury and had lower mercury content. Flower injury was common, however. Spergon, Arasan, and Mersolite 8 caused no plant injury in any location.

Plant-Growth-Regulating Substances Obtained from Cultures of Fusarium moniliforme. MITCHELL, J. E., AND C. R. ANGEL. *Fusarium moniliforme* Sheldon in liquid culture produces a substance which markedly stimulates the elongation of bean plants. This effect is similar to that described in work on the Bakanae disease of rice as reported from Japan. The active material is produced in a cerelose-ammonium sulphate medium. The rapid, diffuse growth of the organism is accompanied by an abrupt drop in the pH of the medium to approximately 2.5. The activity appears when the carbohydrate is nearly exhausted. The active component is weakly acidic, soluble in polar solvents and alkaline solutions. It is stable in acid solutions but inactivated at higher pH levels. Its activity differs from that of indoleacetic acid and 2,4-dichlorophenoxyacetic acid. Application to the bean plant was made by dipping the distal end of a primary leaf petiole into a vial containing the test solution. Six days after treatment plants measured 500 mm. from the axil of the primary leaf to the tip of the terminal infloresence, as compared with 35 mm. for the controls. Flowers and pods on such plants were normal. In

certain preparations of this material a second type of activity has been observed. In these cases, instead of an elongation, a marked twisting and later gall formation occurs on treated plants. Gall formation commonly occurs at the terminal growing point and prevents further development of the plant. Application of this material to plants in the flowering stage resulted in a failure of fruit development.

The Use of Sulfanilamide Derivatives as Eradicant Fungicides for Wheat Stem Rust. MITCHELL, J. E., H. H. HOTSON, AND F. H. BELL. Previous studies have shown that certain sulfonamide derivatives inhibited development of cereal rust when application was made via the soil. The present work confirms and extends these observations to spray applications. When sulfanilamide and several of its derivatives were applied to wheat leaves previously inoculated with stem rust, the development of the rust was markedly inhibited. Of the derivatives tested, sulfadiazine was the most effective; sulfapyridine, sulfanilamide, sulfathiazole and sulfamerazine were less so. The minimum concentration of sulfadiazine that would prevent development of pustules was 0.1 per cent of the sodium salt. The effectiveness of the compounds was limited in part by their penetration into the leaves. By using a wetting agent and placing the plants in a moist chamber for a few hours after treatment the effectiveness of the compounds was increased. The spread between the eradicant and the phytotoxic dosages is not great, 0.4 per cent sulfadiazine retarding plant growth. The compounds appear to inhibit development of rust mycelium. At optimum concentrations no lesions, or at most only flecks, were evident. At less effective concentrations all gradations of pustule development could be observed. Other phytotoxic chemicals such as amate, when used at subphytotoxic levels, prevented the development of rust in the leaves.

The Relative Importance of the Various Control Measures for Sweet-Potato Scurf. NUGENT, T. J. For the past several years various factors in the control of sweet-potato scurf were compared. As a measure of the control obtained, a scurf index, ranging from zero for no scurf to 100 when all potatoes were severely scurfed, was determined at harvest time. The summarized data showed that the scurf index (6-year average) for the harvested potatoes from apparently clean seed was 12.2, and from scurfed seed, 42.1. When bedding mediums were compared using clean seed, sand gave an average scurf index of 22.0, and soil 31.5 (four tests); using scurfed seed, sand 45.1, and soil 45.2 (six tests). When seed treatments were compared, the scurf indexes (average of eight tests with both clean and scurfed seed) were 18.8 for bichloride of mercury plus sulfur, 29.8 for bichloride of mercury, and 34.8 for no treatment. In one similar test, borax plus sulfur gave a scurf index of 23.4, borax 24.2, Semesan Bel (12 per cent hydroxymercurinitrophenol; 3.2 per cent hydroxymercurichlorophenol) 34.2, and no treatment 29.8. In two tests with sprout treatments, sulfur dust gave a scurf index of 14.0, Semesan Bel dip, 13.9, and no treatment, 27.4. For 3 years early plantings averaged 25.9 and late plantings 23.2. In three tests using scurfed seed, sprout cuttings averaged 5.0 and pulled sprouts 49.1.

Cell Proliferations on Irish Potato Slices Caused by Soft-Rot Bacteria. SMITH, WILSON L., JR. Twenty-eight isolates of *Erwinia atroseptica* and 23 of *E. carotovora* were used in these studies. Seventeen of the former species produced cellular proliferation of potato slices, six with regularity. Only seven isolates of *E. carotovora* produced cellular proliferation, one with regularity. Proliferations occurred when the potato slices had been suberized for one or more days or when dilution of the bacterial cells was great enough to cause delay in infection. Proliferations resemble colorless warts or pustules, raised 1–3 mm. above the surface of the potato slice. Microscopically they show a disorganized cellular pattern when young. Cells resembling meristematic cells form more rapidly and deeper in the tissue under the proliferated than under the non-proliferated zone. Older proliferations arising deep in the tissue show long vacuolate cells. Later these raised areas become blackened by a watery exudate from the affected zone. Tests with ether extracts of liquid media in which the respective organisms were growing indicate that an auxinlike substance is produced by both species in equal amounts. Since cellular proliferation occurs more commonly with *Erwinia atroseptica* than with *E. carotovora*, it is believed that factors other than auxin content, as measured in these tests, are active in the production of proliferation.

Flaccidity, Turgidity, and Predisposition in Barley and Wheat as Factors in Susceptibility to Powdery Mildew (Erysiphe graminis DC.). TAPKE, V. F. Recent experiments have shown that when susceptible barleys and wheats are inoculated with powdery mildew, and then exposed to conditions favorable for infection, the mildew attack (in plants beyond the seedling stage) may range from practically zero to 100 per cent depending upon how the plants were conditioned by the environment to which they were exposed before inoculation. Plants grown in the greenhouse or under conditions promoting a tender, succulent growth, are more susceptible than plants grown outdoors or

under cold, hot, dry, or other conditions promoting a toughened growth. This discovery of predisposition appears to aid in clarifying the powdery-mildew-environment relationship in the small grains. For example, Rivera reported that susceptibility in wheat occurs during periods of flaccidity or partial wilting of plants. His inoculated greenhouse-grown plants wilted when first placed under a bell jar and exposed suddenly to sunlight. Later the plants succumbed to mildew. Outdoor-grown plants similarly treated did not wilt or take mildew. The writer finds flaccid and turgid plants equally susceptible. Apparently Rivera's results were due to the use of plants differently preconditioned by environment before inoculation, i.e., hardened outdoor-grown and tender greenhouse-grown plants, wilting of the latter under bell jars being an incidental phenomenon accompanying sudden exposure to the sun's heat.

A Technique for the Collection of Dry Spores From Infected Plants. TERVET, IAN W. A rapid and efficient method of collecting small quantities of spores of certain fungi from infected plants has been devised. The apparatus in its simplest form consists of a small cyclone separator, made of metal or glass, operated in the laboratory and greenhouse by an electrically driven portable blower and vacuum pump (developing about 1.3 cfm.), or in the field by a rubber hand bulb or even by lung pressure. The spores are removed from infected plants by suction; the air, bearing the spores, passes through the cyclone separator where recovery of the particles occurs. By this technique it has been possible to collect conidiospores of powdery mildews, uredospores of rusts, and spores of other fungi from infected plants. Similarly, dry spores of some fungi can be removed from their culture media, and pollen grains and other microscopic plant parts can be readily collected in relatively pure form. Collection has been obtained with spores as small in diameter as 5 to 6.5 μ (*Aspergillus tamarii*), although spore recovery was not efficient. Recovery of larger particles such as uredospores is excellent.

A Simple Method for Collecting and Inoculating Rusts in Race Identification Studies. TERVET, IAN W., AND R. C. CASSELL. Small cyclone separators have proved valuable in studies on cereal rusts. It has been shown that spores from one susceptible pustule or from four to six resistant type-1 pustules of stem rust are sufficient to inoculate the differential varieties for race determination in certain varieties of *Puccinia graminis*. A glass cyclone 8 cm. long and 2 cm. in diameter is partially filled with about 0.5 gm. of talc. Spores are sucked up from the type-3 or type-4 pustule or a few type-1 pustules and collected in the cyclone. Additional talc (about 0.5 gm.) is added to the cyclone, and the spores and talc are thoroughly mixed. By reversal of air flow through the cyclone, the spore-talc mixture is then dusted on the differential varieties. Spores collected from a single susceptible type pustule on field-grown plants, when diluted with talc and so applied, have proved sufficient for direct inoculation of the set of differentials needed for race identification, thus rendering unnecessary prior increase on a susceptible host. Many tests have shown that an average of 1.2 pustules per leaf developed on plants of the wheat stem rust differential series after inoculation with spores from a single pustule and incubation for 12 hr. at 65° F.

Following are titles of other papers presented at this meeting of the Potomac Division:

Serological Diagnosis of European Virus Yellows of Sugar Beet. G. H. COONS and J. E. KOTILA.

A Crown Rot and Wilt of African Violets Caused by Phytophthora sp. CHARLES DRECHSLER and FREEMAN WEISS.

Observations of Plant Diseases in Venezuela. W. F. JEFFERS.

Some Effects of Temperature on Pathogenicity and Growth of Sclerotinia trifoliorum. ERIC O. D. MORGAN.

Preliminary Studies of Peach Brown Rot Canker in Maryland. L. O. WEAVER.

Topcrop, a New Multiple Virus-Resistant Snap Bean. W. J. ZAUMEYER.

Informal discussion of new pathological problems:

Red Leaf Disease of Oats. F. A. COFFMAN.

Stemphylium Blight of Tomato. S. P. DOOLITTLE.

Oak Wilt. CURTIS MAY.

New Fungicides. W. B. MCCLELLAN.

Western Wheat Mosaic. H. H. MCKINNEY.

Demonstrations:

Refrigeration Equipment. H. T. COOK.

Methods of Indexing for Tobacco Disease Resistance. E. E. CLAYTON and J. J. GROSSO.

Phytotoxicity of New Insecticides. A. C. FOSTER.

PHYTOPATHOLOGICAL NOTES

Oat Rust Urediospores Exceed Host Tissue in Cold Resistance. JOHN W. OSWALD AND COIT A. SUNESON. Two factors are important in the carry-over of stem rust during a normal season in California, as has been pointed out by Suneson.[1] First, the growth of volunteer grain in irrigated fields or along ditchbanks carries the rust in the active red stage through the summer and fall and into the winter; and secondly, a mild winter is necessary to insure the viability of the urediospores. Telia usually are formed in the early summer prior to a lengthy hot arid period which renders the teliospores nonviable. Water spilled from an experimental bean field into a depression along a railroad germinated about 100 seeds of *Avena fatua* on an area 6 feet by 30 feet in early September 1948. These plants were observed frequently thereafter. By late December these plants were near the heading stage and were infected liberally with both crown rust (*Puccinia coronata* Cda.) and stem rust (*Puccinia graminis avenae* Eriks. and E. Henn.). Only the uredial (red) stages were present at that time.

The winter of 1948–49 was one of the coldest on record in the Sacramento Valley and some observations of interest on the behavior of oat rusts were made. During the 43-day period from January 3 to February 14, minimum temperatures ranging from 20° F. to 32° F. were recorded on 33 days. The mean minimum temperature during this period was 28.3° F. Freezing temperatures killed all exposed vegetative tissues of the host, and by February 17 only the telial (black) stage of both rusts was apparent on the winter-killed host tissue. On this date, however, new basal growth was appearing on nearly all of the plants.

Late February laboratory inspections of the black stem rust sori showed that some urediospores persisted among the black teliospores. Germination tests demonstrated that the majority of these urediospores were viable and capable of initiating new infections. In the same and later germination tests a small percentage of the teliospores proved to be viable (0.5 per cent in February; 2–3 per cent in March). The viability of the red spores prompted frequent observation of the host plants in this well-isolated site.

On March 22, the first new uredia of both stem and crown rust were seen on the green tissue. On April 7 (a 17-day cycle) the first secondary infections were seen. On April 13, almost 250 stem rust and 25 crown rust pustules were counted. After that date there was no further increase in rust. Owing to the total absence of rain during April, and unusually low humidities, the host plants were dried up or mature on May 20.

The feature of principal interest in these observations probably is the survival of urediospores of both stem and crown rust fungi on frost-killed oat tissue. Further, for the first time viable teliospores of stem rust have been encountered in the central valleys of California. The telia developed

[1] Suneson, Coit A. Year-around occurrence of stem rust of wheat (*Puccinia graminis tritici*) in California. U. S. Dept. Agr., Plant Dis. Rptr. **28**: 410–413. 1944.

during cool weather and thus were able to survive. Also they were chilled sufficiently so that a small percentage of the spores soon broke dormancy.—University of California, Davis, California.

Plaque Formation by Xanthomonas pruni Bacteriophage. A. EISENSTARK AND H. H. THORNBERRY. The activity of *Xanthomonas pruni* bacteriophage, *Phagus pruni* Holmes, occurring on *Xanthomonas pruni* (E. F. Smith) Dowson has been demonstrable mainly by the qualitative phage-lysis technique involving changes in turbidity of broth cultures[1, 2] Although this method suffices for certain uses such as the detection of the phage[2, 3] and the identification of bacterial plant pathogens by specificity of action,[1, 2] it does not provide a means for quantitative assay of the phage and characterization of genetic differences within a population of this phage. Although plaques have been observed on agar in Petri plates and slants,[2, 3] the conditions necessary for their consistent occurrence have not been known. The present studies were undertaken to find a procedure favoring plaque formation similar to that which has been obtained for most bacteriophages.

The bacterial virus used was *Xanthomonas pruni* bacteriophage,[4] and the host bacterium was *Xanthomonas pruni.* The semisolid agar layering method that is generally suitable for the formation of plaques by other phages was modified slightly for this phage. A thick layer of 2 per cent nutrient agar was poured into Petri dishes, 15 ml. per 90-mm. dish, and allowed to solidify. Dilutions of phage were made of young growing *Xanthomonas pruni* cells in nutrient broth in tubes containing 10 ml. final volume. In order to keep these cells in their maximum logarithmic growth phase, cultures were transferred twice daily for at least a week previous to plaque counts. After the phage was mixed with host cells, 1 ml. of each mixture was added to 4 ml. of melted 0.6 per cent agar which was then poured as a thin semisolid layer over the solid layer in the Petri dishes.

Failure to obtain plaque formation in Petri plates poured with nutrient agar mixed with the susceptible organism and active phage prompted us to try other procedures. One difficulty appeared to be related to a "ropy" growth which did not favor a homogenous suspension and resulted in uneven growth throughout the Petri dishes. This was overcome by continuous daily transfer of bacterial cultures to reduce the lag phase to a minimum. When this procedure was combined with that of the semisolid agar layering method, clearly recognizable and well distributed but nonuniform plaques were formed in the bacterial growth over the surface of the nutrient agar (Fig. 1). Three distinct types of plaques predominated within

[1] Thornberry, H. H., A. C. Braun, and R. P. Elrod. Application of the bacteriophage-lysis technique for the identification of plant pathogenic bacteria. Phytopath. 39: 152–154. 1949.

[2] Thornberry, H. H., A. Eisenstark, and H. W. Anderson. Studies on the bacteriophage of *Xanthomonas pruni.* Phytopath. 38: 907–911. 1948.

[3] Anderson, H. W. Bacteriophage of *Bacterium pruni.* (Abstr.) Phytopath. 18: 144. 1928.

[4] No. 1–47 H. H. T., having a 10^{-9} titer when stored May 24, 1947. Culture deposited in Centre de Collection de Types Microbiens, Lausanne, Switzerland.

a plate. A small plaque approximately 1 mm. in diameter and essentially circular with an uneven edge occurred most frequently (Fig. 1, A). A large plaque approximately 3 mm. in diameter and circular with entire margins occurred about one-fourth as frequently as the small plaques (Fig. 1, A). Plaques variable in size and surrounded by a cleared halo appeared in some plates (Fig. 1, B). The cleared halo varied in width from about 0.3 to 2 mm., the most common being about 0.8 mm. These differences in plaques suggest the existence of genetic varieties of the phage, as have been reported for *Escherichia coli* phage. Attempts at isolating varieties of phage were unsuccessful. However, when the phage from single plaques of the halo type was reinoculated to young cells, the resulting plaques were largely of the halo type (Fig. 1, C). This suggests genetic variation.

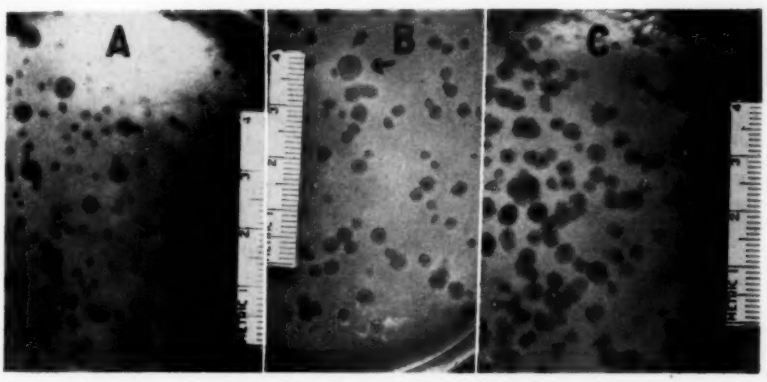

FIG. 1. Plaques produced by *Xanthomonas pruni* phage. A. Small and large types of plaques. B. Plaque with halo. C. Plaques with halos are abundant after an attempt to "purify" this type.

The finding of a procedure for the formation of well-dispersed plaques by this bacterial virus permits quantitative studies of bacteriophage action. A semisolid agar surface and nonclumped bacterial host cells are necessary for the formation of well-dispersed plaques. The variation in plaque characteristics suggests that genetic varieties of strains of this virus may exist and that evidence to this effect may be secured by use of the plaque method. Cleared halos were observed around some of the plaques under the conditions of the quantitative method. These plaques appear to be similar to those reported for *Klebsiella pneumoniae* by Humphries.[5] He attributed the halo effect around plaques to a second factor which he suggested might be an enzyme liberated by the lysing cells. He succeeded in separating the substance from the phage and showed that the two had different properties.—Department of Bacteriology, Oklahoma A. and M. College, Stillwater, Oklahoma, and Division of Plant Pathology, Department of Horticulture, Illinois Agricultural Experiment Station, Urbana, Illinois, respectively.

[5] Humphries, J. C. Enzymic activity of bacteriophage-culture lysates. I. A capsule lysin active against *Klebsiella pneumoniae* Type A. Jour. Bact. **56**: 683–693. 1948.

ANNOUNCEMENT

The Society will hold its 42nd annual meeting at the Peabody Hotel, Memphis, Tennessee, on December 1, 2, and 3, 1950. The Council will meet on November 30.

Abstracts of papers are due in the Secretary's office October 2.

At this meeting a final vote will be taken on a proposed change in the Constitution. At the New York meeting in December 1949 the Council proposed and the Society approved a motion to provide for a President-elect who would become President at the annual meeting next succeeding the annual meeting at which he was elected. This action is included in the Report of the 1949 Annual Meeting, published in the April 1950 issue of PHYTOPATHOLOGY (p. 406). The following revision of the Constitution is needed to effectuate this action.

ARTICLE IV

Section I. The government of the Society and its corporate authority as defined in the Society's Articles of Incorporation shall be vested in a Council which shall consist of the contemporary officers, the retiring President, the President-elect, and the Editor-in-Chief and the Business Manager of the Journal, PHYTOPATHOLOGY, a Councilor from each unit of the Society that is organized as a Division, and two Councilors-at-Large.

Section II. The President shall call meetings of the Council at his discretion or upon the written request of three of its members. He, or in his absence the Vice-President, shall preside at meetings of the Council. In the absence of both the President and Vice-President, the President-elect shall preside at meetings of the Council.

ARTICLE V

Section I. The President-elect, Vice-President, and Councilors-at-Large shall be elected by ballot. (No change in the remainder of this section.)

Section III. The President-elect shall become President and assume the duties of the office upon final adjournment of the annual meeting next succeeding the annual meeting at which he is elected. The Vice-President shall assume office upon final adjournment of the annual meeting at which he is elected. The President and Vice-President shall not be eligible to immediate reelection to the same offices.

CURTIS MAY, *Secretary*